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БОЛЕЗНЬ АЛЬЦГЕЙМЕРА: ВЛИЯНИЕ МИКРОФЛОРЫ КИШЕЧНИКА И ПОЛОВЫХ РАЗЛИЧИЙ НА ПАТОГЕНЕЗ И СТРАТЕГИИ ЛЕЧЕНИЯ

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Alzheimer's Disease: The Impact of Gut Microbiota and Sex Differences on Pathogenesis and Treatment Strategies

Резюме

Болезнь Альцгеймера (БА) — глобальное заболевание. Наиболее важным в патогенезе БА является увеличение отложений амилоидного белка бета (Аβ) и патологическое скопление тау-белка. В развитии болезни принимает участие ряд этиологических факторов, таких как возраст, генетика, образ жизни, факторы окружающей среды и микрофлора кишечника (МК). Нарушение регуляции МК — один из факторов патогенеза БА, который способствует нарушению когнитивной функции, включая скопление Аβ и тау-белка, выработку нейромедиаторов и метаболитов, нарушение иммунной регуляции, нейровоспаление, нарушение гематоэнцефалитического барьера, оксидативный стресс и синдром раздраженного кишечника.

Половые различия могут быть важным фактором патогенеза БА. Около 75 % пациентов с БА являются женщинами. Преобладание БА у женщин связано с генетикой, структурой и функцией головного мозга, эстрогеном, образом жизни (например, образование, род деятельности, уровень физической активности и продолжительность сна) и случаями инфекционно-воспалительных заболеваний. Поскольку продолжительность жизни у женщин больше, чем у мужчин, женщины более склонны к БА.

В настоящей статье рассматривается роль МК и половые различия при БА. В начале статьи приводится краткое описание характеристик микрофлоры кишечника и половых различий при БА. В работе рассматриваются перспективные терапевтические стратегии при БА, направленные на МК.

Ключевые слова: *болезнь Альцгеймера, микрофлора кишечника, половые различия, амилоидный белок бета, тау-белок*

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Abstract

Alzheimer's is a global disease (AD). The most important pathogenesis of AD is the increase in the amyloid-β protein (Aβ) deposition, and abnormal phosphorylation aggregation of the microtubule-associated protein tau. Many etiological factors are implicated in the production of AD such as age, genetics, lifestyle, environmental factors, and gut microbiota (GM). Dysregulation of GM contributes to AD pathogenesis and cognitive impairment via several mechanisms, including Aβ and Tau protein aggregation, production of neurotransmitters and metabolites, immune dysregulation, neuroinflammation, blood-brain barrier disruption, oxidative stress, and leaky gut.

Sex differences might be an important factor for AD pathogenesis. About 75 % of AD patients are females. The higher prevalence of AD in females is due to their genetics, brain structure, and function, estrogen, lifestyle factors (e.g., education, occupation, exercise, and sleep), and incidences of infection and inflammations. Because women live longer than men do, they are more likely to get AD.

This article discusses the role of the GM and sex differences in AD. It begins with an overview of the gut-microbiota axis and sex differences in AD. It discusses promising therapeutic strategies for AD targeting GM.

Key words: *Alzheimer's disease, gut microbiota, sex differences, amyloid-β protein, tau protein*

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AD — Alzheimer's disease, Aβ — amyloid-β protein, APOE — apolipoprotein E, GM — gut microbiota, CNS — central nervous system, HPA — hypothalamic-pituitary-adrenal axis, GABA — gamma amino butyric acid, 5-HT — serotonin, LPS — lipopolysaccharide, TMAO — trimethylamine N- oxidase, SCFAs — short-chain fatty acids, NMDA — ionotropic glutamate receptor, BBB — blood-brain barrier, IL — interleukin, APP — amyloid precursor protein, FSH — follicle-stimulating hormone, BDNF — brain-derived neurotrophic factor, BADLs — Basic activities of Daily Living, IADLs — Instrumental Activities of Daily Living, MRI — magnetic resonance imaging, DASH — Dietary Approaches to Stop Hypertension, GSK — glycogen synthase kinase, FMT — fecal microbiota transplantation

Introduction

Alzheimer's disease (AD) is characterized by deterioration in memory, behavior, thinking, ability to perform daily activities, judgment, and language. It has become a global health epidemic problem. The total estimated prevalence is expected to reach 82 million by 2030 and 210 million by 2050 [1]. Per year, approximately 6 % is the rate of death from AD. The survival duration from the date of AD symptoms is about four years for males and six years for females [2].

There are many hypotheses for describing the pathogenesis of AD including amyloid-β protein (Aβ) deposition, abnormal phosphorylation aggregation of the microtubule-associated protein tau, accumulation of apolipoprotein E (APOE), microglia dysfunction, oxidative stress, neuroinflammation, and astrocyte activation in the gut [3]. Any infections or traumatic brain injury can interfere with central immune homeostasis and accelerate the progression of the disease [4].

A complex combination of aging, genetics, lifestyle, and environmental factors can cause AD. The strongest risk factors for AD are at advanced ages [5]. It affects 50 % of individuals older than 85 %. A strong and statistically significant positive genetic correlation has been observed between AD and family history [6]. Among the environmental factors implicated in AD pathogenesis, rapidly growing evidence from animal and human data suggests an important role of the gut microbiota (GM) in the onset and progression of AD pathology [7]. Additionally, Tan et al., [8] reported that the increasing AD prevalence in recent years was highly correlated with unhealthy diets and environmental exposures that affect the GM composition [9].

There are about 100 trillion commensal microbial communities that colonize the human gut and are constituted by bacteria, fungi, archaea, viruses, and protozoans living in symbiotic relationships with our intestines [10]. The human intestines contain approximately 1000 species and 7000 strains of bacteria which constitute the gut flora [11].

The flora of the intestinal tract is not pathogenic and has numerous beneficial effects on the body's physiological functions and nutrition. For example, intestinal flora participates in energy metabolism, reduces inflammatory response, stimulates systemic immunity, and promotes intestinal motility and nutrient absorption [12].

Animal studies have shown that gut flora regulates memory and learning [13]. Dysregulation of the GM has been associated with abnormal brain protein aggregation, inflammation, immune dysregulation, and impaired neuronal and synaptic activity in animal and human studies of AD [14].

The human GM is influenced by various factors, including genetics, race, mode of delivery (vaginal vs. cesarean), early dietary intake (breastfeeding vs. formula feeding), age, body mass index, medical conditions, psychological factors, acidic pH of the gut, diet, physical activity, stress, lack of sleep and environmental factors [15].

Sex differences may also be a significant factor in addition to these well-known confounding factors. The microbiota compositions before and after puberty were different in the male mice, suggesting male sex hormones may play an important role in the sex differences in GM [16]. When the androgen source was removed by castration, the GM of the castrated male was similar to that of a female mouse. Also, bilateral ovariectomy causes microbial dysbiosis in mice [17] and humans [18]. The GM of postmenopausal women is more similar to that of men than that of premenopausal women [19].

Because there is no treatment for AD, only symptomatic measures, all studies aim to clarify the pathogenesis of the disease for future prevention of the progressive neurodegeneration caused by AD [3].

While there have been extensive studies on GM, research specifically focusing on sex differences and GM in AD is relatively limited with conflicting results. Therefore, this review summarizes the current knowledge of

the mechanistic role of sex differences, GM in the development of AD, and potential gut microbiome-targeting therapies in managing the disease.

Understanding the Microbiota Gut-Brain axis

The gut-brain axis allows a two-way communication network between the intestine and the brain, including the central nervous system (CNS), autonomic nervous system, enteric nervous system, neuroendocrine system, and neuroimmune system [20]. It encompasses several pathways, including the nervous system, endocrine system — hypothalamic-pituitary-adrenal axis (HPA)- and immune system, that work together to regulate various physiology, such as digestion, immune function, mood, cognition, and anxiety [21]. HPA activation leads to a release of cortisol which can cause changes in both GM composition and cognition [22].

The CNS affects intestinal motility, sensory, permeability, and secretion. The GM regulates CNS neurons, astrocytes, microglia, and the blood-brain barrier by the production of a variety of neurotransmitters and metabolites and regulation of inflammation and immune systems [23]. Intestinal flora produces neurotransmitters such as glutamate, gamma amino butyric acid (GABA), serotonin (5-HT), acetylcholine, and dopamine [24]. While gut dysbiosis produces metabolites such as lipopolysaccharide (LPS), trimethylamine N- oxidase (TMAO), short-chain fatty acids (SCFAs) (such as butyrate and acetate), amino acids, and bile acids. It also produces inflammatory cytokines, directly affecting neuro-inflammation or activating peripheral immune cells [25].

Mechanisms of action of GM in driving AD progression

Gut dysbiosis contributes to AD pathogenesis and cognitive impairment via several mechanisms, including Aβ and Tau protein aggregation, production of neurotransmitters and metabolites, immune dysregulation, neuroinflammation, blood-brain barrier disruption, oxidative stress, and leaky gut.

Aβ and Tau protein aggregation

It is widely believed that an increase in the production of Aβ plaques and Tau protein is the most important pathogenesis of AD. The imbalance between production and clearance of Aβ leads to accumulation of it. Aβ is produced by neurons and secreted into the interstitial fluid of the brain. The major clearance system for Aβ and tau proteins is the glymphatic system [26]. Tau protein is a microtubule protein that has a role in neuronal stability. There is a relationship between tau and Aβ. Tau is essential for Aβ action and also Aβ is necessary for tau hyperphosphorylation [27].

Most microorganisms in the human body, including bacteria and fungi, secrete functional amyloid [28]. Bacterial amyloid protein can cross the blood-brain barrier into the blood flow to the CNS, deposit in the brain, and promote Aβ plaques and tau protein accumulation [29]. Furthermore, GM dysbiosis reduces the clearance of Aβ by affecting the gut mucosal barrier and energy homeostasis [30]. GM-induced tau protein aggregation through the TMAO formation and activation of the glycogen synthase kinase 3 beta pathway [31].

Production of Neurotransmitters

1. Glutamate

The excitatory neurotransmitter glutamate is responsible for memory and learning. It has two receptors: metabotropic and ionotropic. The ionotropic glutamate receptor (NMDA) has a role in the AD [32]. Furthermore, the hippocampal NMDA level decreased significantly after antibiotic treatment, indicating that intestinal flora was involved in the metabolic activity of NMDA [33].

2. GABA

Lactobacillus and *Bifidobacterium* are components of normal intestinal microbiota, which can convert sodium glutamate into GABA [34]. There is cognitive and memory impairment when the function of the GABA system is impaired. GABA also participates in the proliferation of precursor neurons, synaptic formation, and inhibition of inflammation *in vivo* [35].

3. 5-HT

It is a neurotransmitter produced by the gastrointestinal tract chromaffin cells [36]. *Candida*, *Streptococcus*, *E. coli*, and *Enterococcus* indirectly stimulate intestinal cells to store and release 5-HT [35].

It influences mood, memory, and overall bodily functions as a stimulant. Thus, disturbances in 5-HT metabolism coming about because of uneven characteristics in the gastrointestinal microbiota may assist the movement of neurodegenerative problems [37].

4. Acetylcholine

The expression and functioning of acetylcholine are closely associated with AD [38]. Acetylcholine is a commonly occurring metabolite in bacteria, specifically in *Lactobacillus plantarum*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* [39]. Nevertheless, acetylcholine is unable to traverse the blood-brain barrier (BBB); however, its precursor choline can be transported to the brain through a carrier present on the capillary endothelial cells. Once in the brain, choline can contribute to the biosynthesis of acetylcholine [40].

5. Dopamine

Dysregulation of the dopamine system significantly contributes to the pathological progression of AD [41]. Staphylococcus bacteria residing in the human gut produce substantial quantities of dopamine via the enzymatic activity of aromatic amino acid decarboxylase [42]. The disruption of the dopamine system due to alterations in the GM has the potential to expedite the pathological progression of CNS disorders, including AD [37].

Production of Metabolites

As discussed before, GM can produce bioactive metabolites. These metabolites can cross the BBB to affect cognition directly or indirectly through immune, neuroendocrine, or vagal mechanisms [25].

1. LPS

It is the glycolipid formed by the combination of lipids and polysaccharides. Contrasted and the age-matched control bunch, the typical LPS level of the cerebral neocortex in old Promotion patients expanded multiple times. Neuroinflammation of AD patients may be determined by LPS [43].

LPS causes neuroinflammation, microglia activation, an increase in the permeability of the intestine, and changes in BBB [44]. Animal experiments also confirmed that intraperitoneal injection of LPS could increase the Aβ protein level in the hippocampus of mice, resulting in learning disabilities [45].

2. TMAO

It promotes neuro-inflammation and the accumulation of Aβ and tau proteins by inducing the imbalance of intestinal microorganisms. Additionally, it induces the release of proinflammatory mediators [46].

TMAO causes neurodegeneration by affecting fragile neurons, brain, and neuronal aging, increases oxidative stress damages mitochondrial function [47], and can lead to cognitive impairment. Therefore, anti-TMAO preparations can inhibit the course of AD [48].

3. SCFAs

SCFAs participate in nerve conduction and regulate cognition and behavior. Butyric acid and propionic acid can promote tyrosine and tryptophan hydroxylase expressions, which are involved in synthesizing dopamine, norepinephrine, and 5-HT [49].

4. Amino acid

Neural function in the AD brain is greatly impacted by glutamate metabolism [50]. The decrease of Tryptophan reduces 5-HT and leads to cognitive decline [51].

Similarly, gut bacteria have an effect on tyrosine and valine metabolism in the diet. Tyrosine is a precursor of the catechol neurotransmitters dopamine, norepinephrine, and epinephrine. These tyrosine-dependent neurotransmitters affect various central and peripheral functions, which are involved in stress response and working memory [52]. Reduced plasma valine concentrations are linked to faster cognitive loss, and individuals with AD have much lower valine concentrations. On the other hand, as the brain absorbs valine more readily than other branched-chain amino acids, higher valine concentrations can lower the risk of AD [53].

5. Bile acid (BA)

It can also be produced in the brain or transferred from the peripheral circulation to the brain via BA transporters via the BBB. BA influences cognition, memory, and motor skills [54]. Kiriyama and Nochi [55] investigated the relationship between intestinal microbiota, BA distribution, and genetic variation in AD etiology. Conjugated BA Tauroursodeoxycholic acid (TUDCA) has been found in tests to decrease Aβ peptide buildup in the hippocampus and frontal cortex, leading to improved memory. Therefore, they have protective effects on nervous system diseases [55].

Immune Dysregulation

Activated astrocytes are supportive cells that affect neuroinflammation in AD and supply nutrients and metabolic support for neurons [56]. To remove the accumulated Aβ, astrocytes also release chemokines and pro-inflammatory cytokines. A positive feedback loop is created by the extra Aβ deposition, which encourages astrocyte activation and increases the release of pro-inflammatory cytokines. Massive pro-inflammatory cytokine production can harm microglia, impair their capacity to remove toxic Aβ, hinder their capacity to repair synapses and cause irreparable brain damage [57].

The creation of gut-associated lymphoid tissue plays a role in priming the innate immune system, and the gut microbiota also regulates adaptive local and systemic immune responses. Alterations to the gut microbiome are associated with increased penetration of peripheral T-helper1 immune cells into the BBB, increased microglial activation, Aβ aggregation, and cognitive decline in AD mouse models [58].

Gut and Neuroinflammation

AD is characterized by systemic and gut inflammation. It is associated with an increase in inflammatory markers such as interleukin1 (IL1), IL6, IL12, and IL18, interferon, and tumor necrosis factor leading to neuronal cell death and ultimately Aβ and tau protein deposition [59].

On the other hand, intestinal flora is closely associated with gut and neuroinflammation [60]. Microbiota-host immune interactions in the gut lead to the release of proinflammatory mediators, e.g., cytokines such as IFN-γ, IL-1β, IL-6, and TNF-α and other inflammatory

mediators, and specific antibodies involved in the regulation of brain immunity [61]. The increase in gut pro-inflammatory is accompanied by enhanced systemic inflammation and neuroinflammatory processes [48].

In addition, microbial disorders at the intestinal level may damage intestinal permeability and induce systemic activation of the immune system [46]. Patients with AD have markedly higher levels of calprotectin in their brains and CSF, a marker of intestinal inflammation. It implies that intestinal permeability could be associated with AD pathogenesis [62]. Gut inflammation might also have sex differences concerning the GM. In a mouse model of colitis induced with 2,4,6-trinitro benzenesulfonic acid, the males exhibited more severe colonic inflammation [63].

Blood-brain barrier disruption

Even in the early stages of AD, disruption of the BBB has been preset. This disruption is linked to increased amyloid pathology because it impairs the clearance of Aβ, as well as the loss of pericytes and endothelial tight junctions [64]. Aβ and tau aggregation or neuronal death may be preceded by BBB changes, according to animal studies [65].

On the other hand, gut dysbiosis is associated with increased BBB permeability in animal studies which improves after restoring gut microbial homeostasis [66].

Oxidative stress

Under stressful conditions, reactive oxygen species formation increases within mitochondria and increases the risk of developing AD. Oxidative stress increases tau hyperphosphorylation and Aβ accumulation in AD, which leads to the eventual loss of synapses and neurons [67].

Because gut dysbiosis affects the CNS's levels of oxidative stress, it may play a role in the development of AD. For example, NO conversion from nitrate and nitrite by Lactobacillus, E. coli, and Bifidobacterium increases the permeability of the BBB and contributes to neurotoxicity in AD [68]. Pathogenic enteric bacteria, such as Salmonella and E. coli, may cause the stomach to produce hydrogen sulfide, which lowers mitochondrial oxygen consumption and increases the expression of pro-inflammatory cytokines [69]. The primary source of hydrogen, a highly diffusible bioactive gas, is anaerobic cocci which are members of the Enterobacteriaceae family. Reduced hydrogen synthesis and restricted gas availability to CNS neurons may result from gut dysbiosis [70].

Leaky gut

The condition known as inflammation is linked to the breakdown of the intestinal epithelial barrier, which makes it easier for endotoxins, inflammatory cells, and germs to enter the bloodstream [71]. While certain gut species such as Lactobacillus plantarum, Escherichia coli Nissle, and Bifidobacterium infantis enhance the expression of tight junction proteins, others such as the Bacteroides fragilis toxin disrupt the intestinal barrier [72]. Serum samples from individuals with dementia have increased markers of gut permeability, such as serum diamine oxidase levels, and increased inflammatory mediators including the soluble cluster of differentiation 14 levels compared to controls [73].

The cross-talk between AD and sex- differences

SEX DIFFERENCES IN THE INCIDENCE OF AD

Two-thirds of AD patients are women, and women have a greater lifetime risk of developing AD (1 in 5) compared with men (1 in 10) [74]. Sex-specific differences in genetics, race, brain structure, and function, sex hormones, traumatic brain injury, infection and inflammations, and lifestyle factors (e.g., education, occupation, exercise, and sleep) may contribute to AD development. Females have a higher frequency of AD due to their greater longevity than males [75].

Genetic factor (The APO E gene)

The gene of APOE is present on chromosome 19 and there are three alleles (ε2=8 %, ε3=77 %, and ε4=15 %). APOE ε4 is associated with AD [76]. The effect of the APOE ε4 genotype is more pronounced in women than in men [77]. AD risk increases nearly 4- and 10-fold in women with one and two APOE ε4, whereas men exhibit essentially no increased risk with one APOE and a four-fold increased risk with two APOE ε4 [78].

Race

In general, older Hispanics and African Americans are more likely to get AD than older whites [79]. Differences in health, lifestyle, and socioeconomic status are believed to contribute to their increased risk of AD. These include a higher prevalence of CVD, T2DM, hypertension, and early childhood adversity, as well as low education and physical activity [80].

Brain structure and functions

Head size and cerebral brain volume are 10 % larger in men than in women [81]. Also, women have a higher percentage of grey matter and hippocampus, whereas men have a higher percentage of white matter, amygdala, and thalamus. These sex differences contribute to performance differences. In particular, men perform better on visually oriented tasks, while women perform better on verbal memory [82].

Hormonal factor

Estrogen is protective against AD pathology. It reduces levels of Aβ by stimulating the generation of amyloid precursor protein (APP)-containing vesicles from the Golgi network, thereby promoting APP delivery to cell surfaces [83].

It has been shown to play a role in emotions, memory, and cognitive functions. Several studies have shown a higher incidence of AD in females after menopause [84]. Females who started hormone replacement treatment within 5 years after menopause had a 30 % reduced incidence of AD than women who did not utilize hormone replacement therapy [85].

In addition to estrogen and testosterone, other hormones, including oxytocin, prolactin, and follicle-stimulating hormone (FSH), have been implicated in processes related to AD. Oxytocin and prolactin may be involved in neuroprotection and the regulation of inflammation [86]. Elevated FSH levels are associated with lipid metabolism, obesity, and cognitive deterioration in menopausal women. The blockade of FSH improved cognition in mice with AD [87].

Traumatic Brain Injury

There is a link between traumatic brain injury (TBI) and an increased AD risk [88]. Compared to their male counterparts, females are far more likely to experience worse outcomes, more severe symptoms, and a slower pace of recovery after moderate traumatic brain injury and concussions [89]. Estrogen administration pre- and post-TBI is associated with increased neuronal survival, significant reductions in apoptosis, and improvements in functional outcomes [90].

Infection and Inflammation

It has been shown that there are sex differences in the way that infections and inflammation are responded to and experienced; in particular, when there is a decrease in estradiol levels, females tend to have more severe illness and poorer prognoses than males [91]. For example, especially after menopause, women are more likely to develop chronic inflammatory diseases such as multiple sclerosis, lupus, and rheumatoid arthritis [92].

Lifestyle Factors

A sedentary life is associated with a higher risk of dementia and greater cognitive decline among older adults [93]. Women tend to engage in less physical activities than men. It has been demonstrated that increased physical activity increases the synthesis of brain-derived neurotrophic factor (BDNF), which is crucial for the development, growth, and plasticity of neurons, as well as the creation, survival, and synaptic plasticity of new neurons in the hippocampus [94].

A higher risk of AD is linked to low levels of occupational and educational performance. More education and mentally demanding jobs increase one's cognitive reserve. Women in low-income nations are less likely than males to have access to schooling, which may have negatively impacted their ability to accumulate cognitive reserve [95]. When compared to men, women are generally at a greater risk for sleep deprivation and insomnia, especially after menopause. Sleep deprivation leads to an increase in Aβ plaque accumulation [96].

SEX DIFFERENCES IN SYMPTOMS

Female patients were more frequently to show cognitive and functional decline, depression, delusion, and memory impairment including verbal learning, delayed recall, and visual memory [97]. While males were more likely to exhibit indifference, anxiousness, and hostility [98].

A meta-analysis of 15 studies revealed a consistently better performance in males over females on verbal, visuospatial, episodic, and semantic memory independent of age, education level, and disease severity [99]. However, it has been reported that premorbid depressive symptoms, significantly increased the risk for dementia, particularly AD in men but not in women [100]. Women seem to be more susceptible to pathological lesions while men have greater cognitive reserve [101].

SEX DIFFERENCES IN DIAGNOSIS

A study examined Aβ and tau levels in the brain by positron emission tomography scanning in 298 cognitively normal-aged men and women found that women had higher levels of AD pathology, despite not having symptoms. This shows that while women may be more susceptible to the development of AD pathology and symptoms, there may be sex-specific characteristics that compensate for the early stages of the illness [102]. In example, cognitive impairment in women is linked with bigger reductions in fluency capability, whereas in males it is associated with considerable declines in visual-spatial ability. In women, the intensity of delirium was connected with dementia [103].

In women, delirium severity was related to dementia severity. For men, unlike for women, delirium severity was greater in those with lower educational levels. Differences were noted based on gender and race. African American women reported greater difficulty with all Basic activities of Daily Living (BADLs) and Instrumental Activities of Daily Living (IADLs) except dressing and using the telephone. In comparison to males, non-Hispanic White women reported considerably more difficulty with transfers, indicating a gender gap in this mobility-related daily activity. African American men and non-Hispanic White men demonstrated an equivalent prevalence of difficulty for all BADL tasks. However, for all IADLs African American men reported greater difficulty compared to non-Hispanic White men [104].

Studies using magnetic resonance imaging (MRI) showed greater loss of gray matter in brain regions, including the bilateral precuneus, caudate nucleus, entorhinal gyrus, thalamus, middle temporal gyrus, insula, and amygdala in women with AD compared to men [105]. Furthermore, neuroimaging studies showed that the rate of hippocampal atrophy affects the progression of AD in females more than in males. A neuroimaging study showed that post-menopausal women exhibited higher tau and global Aβ deposition than men in the inferior parietal, rostral middle frontal, and lateral-occipital regions compared to age-matched men [106].

Potential Therapeutic Strategies for AD Targeting the Microbiota-Gut-Brain Axis

Dietary modification

Mediterranean diet is associated with a high input of fruits, vegetables, cereals, and legumes; and a low input of meat, high-fat dairy, and sweets [107]. It's characterized by bettered cognition, reduced brain atrophy in regions vulnerable to announcement pathology, advanced tube carotenoid situations and paraoxonase exertion, advanced SCFA situations, increased gut microbial diversity, and lower supplemental labels of inflammation (e.g., C- reactive protein) [108].

Reduced inflammation and oxidative stress in the brain, and high situations of fiber, vitamin C, β- carotene, and folate are the neuroprotective mechanisms of these diets. As a result, it improves brain integrity and increases the quantum of brain towel [109]. It has also been reported that impregnated and trans adipose acid insufficiency may reduce BBB dysfunction and amyloid aggregation [110].

Also, the high input of fiber, vitamins (e.g., B1, B9, and B6), and minerals (copper, manganese, magnesium, iron, and potassium), were associated with bettered cognition and reduced frailty in another study [111]. Salutary rudiments rich in Vitamin D3 (e.g., dairy and fish) promote the neural growth factor protein [112], and those rich in flavonoids (e.g., grapes, citrus, and green tea) or the polyunsaturated adipose acid, docosahexaenoic acid (e.g., fish) may reduce Aβ and tau pathology and neuroinflammation [113].

Analogous diets, similar to the Dietary Approaches to Stop Hypertension (DASH) diet, also have salutary goods on brain health when combined with exercise [114]. Diets that combine rudiments from both the Mediterranean and DASH diets, which are rich in fruits, vegetables, whole grains, low-fat dairy, and spare protein, may be more effective in delaying cognitive decline [115].

The ketogenic diet also has salutary goods in brain health. In announcement mouse models, ketones reduce oxidative stress, help intracellular uptake of Aβ, and ameliorate synaptic malleability [116]. In mice models, ketone bodies have been demonstrated to influence neurotransmission, reduce neuroinflammation and oxidative stress, as well as reduce Aβ accumulation, and ameliorate literacy and memory capacities [117]. likewise, the ketogenic diet has been shown to alter the gut microbiome, reduce announcement pathology and ameliorate cognition [118].

The combination of the Mediterranean and ketogenic diets is associated with increased SCFA product by GM, bettered CSF labels of Aβ and tau, and better cognitive performance [119].

Table 1. *Potential therapeutic strategies for ad targeting the microbiota-gut-brain axis*

Therapeutic strategies	Mechanism	Refs
1. Dietary Modification:		
Mediterranean diet	It enhances cognition and gut microbial diversity. It also reduces brain atrophy, BBB dysfunction, amyloid aggregation oxidative stress and neuroinflammation.	108, 109, 110
Ketogenic diet	It reduces neuroinflammation, Aβ accumulation and oxidative stress. It helps intracellular uptake of Aβ, and ameliorate synaptic malleability	116, 117
Intermittent fasting	It promotes hippocampal neurogenesis through activation of GSK-3β and increased BDNF, increase insulin perceptivity, reduce inflammation, and promote autophagy	120
2. Antibiotics	It reduces the intestinal microflora, microglial exertion and pro-inflammatory cytokines.	122, 123
3. Prebiotics	It enhances cognitive and memory functions, butyrate levels, production of SCFAs, restoring the balance between anti- and pro-inflammatory bacteria in the GM, insulin sensitivity and production of nerve growth factor and BDNF. It also reduces Aβ accumulation, restoration of redox homeostasis and neuroinflammation.	127, 128, 42, 130
4. Probiotics	It enhances cognitive and memory functions , immunomodulation, long-term potentiation, and intestinal epithelial barrier and BBB functions. It also reduces neuroinflammation, Aβ accumulation and oxidative stress.	134, 136
5. Fecal microbiota transplantation	It enhances cognitive and memory functions, synaptic plasticity and boosted SCFA-producing gut microbes. It reduces neurogenesis, memory impairment, inflammatory cytokines, and Aβ plaque formation.	123, 108

Intermittent fasting has also been shown to promote hippocampal neurogenesis through activation of glycogen synthase kinase (GSK)- 3β and increased BDNF, increase insulin perceptivity, reduce inflammation, and promote autophagy and protein concurrence in beast studies [120]. A 30 reduction in calories from carbohydrates averted Aβ shrine accumulation in a model of AD complaint in womanish, but not manly mice [121], which was associated with coitus-specific changes in amyloid-precursor processing enzymes.

In summary, salutary interventions are generally safer and further salutary than medicine remedies because they're affordable, easy to administer, and reduce the burden on caregivers of announcement cases.

Antibiotics

Antibiotics can affect AD by changing the intestinal microflora. DNA analysis of the cecum and feces of mice treated with antibiotics showed that Aβ shrine deposit was significantly reduced and could restore intestinal microflora analogous to that of the control group. Likewise, intestinal permeability was also restored, and glial cell reactivity in the original area of the shrine was weakened [122]. It also reduced microglial exertion and proinflammatory cytokines similar as IL- 1β and IL- 17A in manly, but not womanish [123].

Ceftriaxone use can reduce the increase of glutamate by perfecting glutamate transport, which is generally present in the area of Aβ shrine deposit, thereby perfecting neuronal activity in mice [124].

Still, some antibiotics (similar to streptozotocin and ampicillin) can disrupt the intestinal bacteria balance [125]. The use of these antibiotics is conducive to or worsens the course of disease. Such as, rats taking ampicillin have elevated glucocorticoids, increased anxiety and worse spatial memory. The increase in glucocorticoids is related to memory impairment and dropped hippocampal BDNF, common features of AD pathology. Ampicillin treatment also significantly depresses the action of NMDA receptors in the hippocampus of rats [126].

Prebiotics

Prebiotics are short-chain carbohydrate substances able to widely stimulate the growth and/ or activity of one or more beneficial gut bacteria [127]. They have also decreased Aβ accumulation, restoration of redox homeostasis, and increased butyrate levels [42].

Yeast beta-glucan has elevated the production of SCFAs, restoring the balance between anti — and proinflammatory bacteria in the GM and reduced neuroinflammation [128].

Mannan oligosaccharides have improved cognitive and memory functions, enhanced synthesis of SCFAs, reduced accumulation of Aβ in the cerebral cortex, hippocampus, and amygdala, as well as reduced neuroinflammation [42].

Lactulose has been shown to reduce neuroinflammation, promote insulin sensitivity, and improve short-term memory and learning [129].

Ferulic acid has anti-inflammatory and anti-oxidant effects and increases the production of nerve growth factor and BDNF [130].

The effect of prebiotics was also different between the sexes. The administration of oligofructose increased the abundance of Bacteroidetes in female rats though the butyrate levels were increased, but not in males, [131].

Still, other authors suggest that further substantiation for the use of prebiotics in clinical practice is still demanded for concluding the normalization of several factors such as age, gender, race, and diet [132].

Probiotics

It is the live microorganisms that change microbiota toward a beneficial state **[133].** Probiotic supplementation causes improvement of immunomodulation, long-term potentiation, and intestinal epithelial barrier and BBB functions [134]. Mice treated with probiotics showed increased memory and significantly lower quantities of plaques and neuroinflammation [135].

Probiotics have anti-inflammatory, anti-stress, and anti-oxidant effects in humans [136].

The effect of probiotics was also different between the sexes, they lowered the colonic mucosal mast cell count and decreased the levels of inflammatory cytokines only in females but not in males [137].

A mixture of a probiotic plus a prebiotic, and synbiotic supplement improved memory, visual-spatial, executive, and linguistic abilities in test subjects and decreased the formation of proinflammatory cytokines (IL-8, IL-12, and TNF-α) [138].

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is the technique of introducing prescreened feces into patients' GI tracts to restore function and boost the total variety of GM [139]. Fecal material is expected to come from a well-organized stool bank and be administered via colonoscopy, enema, or capsule [140].

Dodiya et al. [123] found that FMT enhanced cognition, lowered Aβ buildup and tau expression, improved synaptic plasticity, and boosted SCFA-producing gut microbes. Transplanting feces from AD model donor mice into healthy mice led to decreased of neurogenesis, memory impairment, inflammatory cytokines, and Aβ plaque formation [108].

Conclusion

AD is a global health crisis. The gut-brain axis controls several aspects of brain and gut physiology. Through a number of pathways, gut dysbiosis contributes to the pathophysiology of AD and cognitive decline. It results in the aggregation of Tau and Aβ proteins,

immunological dysregulation, neuroinflammation, disruption of the blood-brain barrier, oxidative stress, and leaky gut. Sex differences may have a major impact on GM. Women make up two thirds of AD patients, and they are more likely than males to have AD during their lifetime. AD treatment strategies that target the gut-brain-microbiota axis include dietary changes. These strategies may be more effective when combined with a high-fiber, vitamin- and mineral-rich diet. Intermittent fasting combined with a ketogenic diet activates GSK-3β and increases BDNF to support hippocampal neurogenesis. Probiotics, prebiotics, and fecal microbiota transplantation might all be important.

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ОСОБЕННОСТИ ВЕДЕНИЯ БОЛЬНЫХ С НАЖБП И САРКОПЕНИЕЙ

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Features of Management of Patients with NAFLD and Sarcopenia

Резюме

С учетом роста заболеваемости неалкогольной жировой болезнью печени (НАЖБП) и демографического старения населения саркопения стала рассматриваться как одно из патологических состояний, участвующих в развитии и прогрессировании НАЖБП. Установлено, что НАЖБП и саркопения связаны с резистентностью к инсулину и атрофией скелетных мышц как органа-мишени инсулина. В патогенезе НАЖБП участвуют воспалительные цитокины с последующим распадом белков скелетных мышц, адипонектин, низкий уровень которого приводит к снижению чувствительности к инсулину, оксидативный стресс с активацией катаболических процессов и развитием атрофии мышц. Прогрессирование саркопении при НАЖБП является прогностическим фактором и увеличивает риск смертности. Саркопения, преимущественно возникающая за счет снижения мышечной массы, в сочетании с увеличением висцерального жира, достаточно часто приводит к саркопеническому ожирению и при НАЖБП увеличивает риск сердечно-сосудистых заболеваний. Гипераммониемия, аномальная микробиота кишечника, липидные факторы также способствуют развитию саркопении у пациентов с НАЖБП. Учитывая общие патогенетические механизмы, указывающие на двунаправленную связь между саркопенией и НАЖБП, мультидисциплинарный подход к ведению больных с НАЖБП и саркопенией в настоящее время представляется наиболее оптимальным. Современные концептуальные стратегии направлены на раннюю диагностику НАЖБП и саркопении, оздоровление образа жизни таких больных, поиск эффективных лекарств, комплексное персонализированное лечение, профилактику прогрессирования данных заболеваний и их осложнений.

Ключевые слова: *неалкогольная жировая болезнь печени, саркопения, ожирение, мышечная масса, мышечная сила, мышечная функция*

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Abstract

Nowadays non-alcoholic fatty liver disease (NAFLD) and sarcopenia are actually considered as one of the pathological condition with involvement into development of pathology of liver and skeletal muscles. Scientists of different countries found that sarcopenia is associated with insulin resistance and skeletal muscle atrophy as an insulin target organ. Spredly known many cytokines of inflammation with variable affection of skeletal muscle proteins, low level of adiponectin, decreased insulin sensitivity, oxidative stress with activation of catabolism leading to muscle atrophy are involved into complicated pathogenesis of NAFLD. Progressive sarcopenia associated with NAFLD is prognostic factor and can increase the risk of mortality. Sarcopenia, which due to decreased skeletal muscle mass and increased visceral fat, very often provokes development of sarcopenic obesity and NAFLD. Hyperammonemia, abnormal intestinal microbiota, lipid factors also contribute to the development of sarcopenia in patients with NAFLD. Given the common pathogenetic mechanisms indicating a bidirectional relationship between sarcopenia and NAFLD, a multidisciplinary approach to

the management of patients with NAFLD and sarcopenia could be the most optimal. Modern strategies are aimed at early diagnosis of NAFLD with sarcopenia, optimizing the lifestyle of these patients, searching for effective drugs, personalizing treatment and prevention of the progression of these diseases and their complications.

Key words: *non-alcoholic fatty liver disease, sarcopenia, obesity, skeletal muscle mass, muscle strength, muscle function*

Conflict of interests

Co-author of the article Statsenko M.E. is a member of the editorial board of the journal «The Russian Archives of Internal Medicine». The article passed the journal's peer review procedure. Statsenko M.E. was not involved in the decision to publish this article. The authors did not declare any other conflicts of interest

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AOP — antioxidant protection, ROS — reactive oxygen species, WHO — World Health Organization, HCC — hepatocellular carcinoma, DHEA — dehydroepiandrosterone, IR — insulin resistance, CT — computed tomography, CPK — creatine phosphokinase, HDL — high-density lipoproteins, MS — metabolic syndrome, MRI — magnetic resonance imaging, NAFLD — non-alcoholic fatty liver disease, NASH — non-alcoholic steatohepatitis, LPO — lipid peroxidation, RCTs — randomised controlled trials, DM2 — type 2 diabetes mellitus, TG — triglycerides, UDCA — ursodeoxycholic acid, LC — liver cirrhosis, ANGPTL4 — angiopoietin-like protein 4, ASM — appendicular skeletal muscle mass, ASMM — appendicular skeletal muscle mass measurement, BIA — bioelectrical impedance analysis, CRP — C-reactive protein, CX3CL1 — monocyte chemoattractant protein 1, DXA — dual-energy X-ray absorptiometry, EMA — European Medicines Agency, EWGSOP — European Working Group on Sarcopenia in Older People, FDA — Food and Drug Administration (USA), IGF1 — insulin-like growth factor 1, IL-6 — interleukin 6, SARC-F — Strength, Assistance with Walking, Rising from Chair, Climbing Stairs, and Falls, SARM — synthetic androgen receptor modulators, SMM — skeletal muscle mass, SPPB — Short Physical Performance Battery, TNF-α — tumor necrosis factor α, TUG — walking time test, TWEAK — TNF-like weak apoptosis inducer, VEGF — vascular endothelial growth factor

This article represents the analysis of literature in ELibrary, PubMed reference databases concerning publications in 2007–2023.

Non-alcoholic fatty liver disease (NAFLD) is currently defined as the most common chronic liver disease that includes steatosis, non-alcoholic steatohepatitis (NASH) with fibrosis, liver cirrhosis (LC), and hepatocellular carcinoma (HCC). Prior studies have demonstrated that NAFLD increases the risk of cardiovascular diseases, cancer, type 2 diabetes mellitus (DM2) [1–3]. Sarcopenia has been deemed the progressive disease associated with DM2, metabolic syndrome (MS), liver diseases, and cardiovascular diseases [6–8]. A common combination of NAFLD and sarcopenia can be considered two interdependent conditions associated with aging, systemic inflammation, and insulin resistance (IR) [8].

The term „sarcopenia" meaning age-related muscle mass loss was introduced into practice by I. Rosenberg in 1989. In 2000, R.N. Baumgartner proposed the term „sarcopenic obesity", i.e. the condition characterised by the combination of excessive adipose tissue in the body and decreased muscle amount, decreased muscle strength, and impaired muscle function [5].

In 2010 and 2019, European Working Group on Sarcopenia in Older People (EWGSOP) developed and published diagnostic criteria, which made sarcopenia a widely renowned disease [6, 7].

The prevention of sarcopenia and its detection in the presarcopenia stage in patients with NAFLD is an up-to-date objective of modern medicine. It has been shown that the risk of NAFLD in patients with sarcopenia is increased more than 5-fold, regardless of the presence of obesity [8, 9]. S. Petta et al. detected a linear incremental growth of the sarcopenia & fibrosis (especially severe fibrosis, F3–F4) severity association. Significant associations have also been proven for sarcopenia and NASH, sarcopenia and steatosis severity. The prevalence of sarcopenia in the European population with hepatic fibrosis depending on its severity is as follows: F0 — 22.2 %, F1 — 34.9 %, F2 — 43.7 %, F3 — 66.6 %, F4 — 60.0 %. With that, the prevalence of severe fibrosis along with sarcopenia was higher both in patients with visceral obesity (46 % vs. 30.9 %) and in patients without obesity (44.4 % vs. 7.1 %, respectively) [10].

The association between sarcopenia prevalence and steatosis severity has been detected in the Asian population. Sarcopenia was observed in healthy subjects from the control group without NAFLD (~8–22 %), in patients with NAFLD (~18–38 %) and NASH (~35–63 %). A high incidence of NASH with fibrosis (46 %) was also detected in patients with sarcopenia compared to those without it (25 %), as well as with a higher risk of NASH (2.5-fold) and significant fibrosis in patients with NAFLD, regardless of obesity and IR [11–14].

Diagnostic aspects of NAFLD and sarcopenia

In NAFLD, fat is detected in over 5 % of hepatocytes in patients not abusing alcohol (< 20 g/day for females, < 30 g/day for males), while the severity varies from simple steatosis to steatohepatitis, progressive fibrosis and LC. Liver biopsy is currently considered the „golden"

standard of NAFLD diagnosis despite limitations concerning the sample variability, invasiveness, and high costs.

Multiple non-invasive biomarkers, serum markers, imaging methods are mainly intended to detect steatosis, NASH, or severe fibrosis. Currently US examination is proposed as a screening method for the diagnosis of steatosis in the target population, while the diagnosis of NAFLD requires the exclusion of other steatosis causes in chronic liver diseases. Progressive fibrosis is important in the NAFLD diagnosis; this can be excluded using the NAFLD Fibrosis Score, FIB-4 score, or with transient elastography. The most reliable diagnostic methods are represented by magnetic resonance imaging, enabling the accurate steatosis evaluation or determining the fibrosis stage, although they are still not applicable in routine practice [3].

The updated consensus document EWGSOP2 presented in 2019 defines sarcopenia as a progressive and generalised disease of skeletal muscles characterised by the decreased muscle strength, mass, and physical ability. The main attention is paid to low muscle strength as a key criterion for the diagnosis of sarcopenia, with the addition of evaluating muscle amount and quality to confirm the diagnosis. Severe sarcopenia is diagnosed in the presence of all three criteria: low muscle strength, low muscle amount or quality, and decreased physical ability. Sarcopenia is divided into acute and chronic forms: an acute condition spans for less than 6 months, while the chronic form lasts ≥ 6 months [6, 7].

In clinical practice, the diagnosis may start from the detection of symptoms, i.e. frequent falls, weakness sensation, slowed walking rate, and difficulties when rising from a chair. In such cases, further testing for sarcopenia is recommended. To screen for sarcopenia, EWGSOP2 proposes the SARC-F (Strength, Assistance with Walking, Rising from Chair, Climbing Stairs, and Falls) questionnaire, enabling patients to assess their limitations of daily activities independently. The questionnaire consists of five items and serves as a simple tool for the detection of sarcopenia risk in clinical conditions. EWGSOP2 specifically denotes a high SARC-F validity, its sensitivity from low to moderate, and very high specificity for the prognosis of low muscle strength with ≥ 4 points [6, 7].

As an alternative, a more formal tool is proposed for the detection of sarcopenia cases in clinical populations — this Ishii test is based on three variables: age, grip force, and calf circumference. Functional tests are more informative in the muscle strength assessment. „Rise from a chair" test defines the time within which a patient can rise from a chair five times without using arms (it is usually over 15 s in sarcopenia) [6, 7].

Based on EWGSOP2 guidelines, the amount of skeletal muscles is assessed as their total mass (SMM), as well as the mass of appendicular skeletal muscles. Magnetic resonance imaging (MRI) and computed tomography (CT) are defined as a golden standard for the non-invasive evaluation of the skeletal muscle amount or mass according to the latest EWGSOP2 guidelines; nevertheless, in the majority of cases these methods are not widely used due to high equipment costs and the absence of highly qualified staff. As noted by EWGSOP2, dual-energy X-ray absorptiometry (DXA) is more frequently preferred to measure the appendicular skeletal muscle mass (ASMM) [6, 7].

Normal values for the appendicular skeletal mass (ASM) (DXA) are as follows: males > 7.26 kg/m^2, females > 5.76 kg/m^2.

Bioelectrical impedance analysis (BIA) does not measure the muscle mass directly, but rather assesses the muscle mass based on the electric conductivity of the whole body [6, 7].

Physical ability may be tested using the 4-meter walking speed, Short Physical Performance Battery (SPPB), walking time test (TUG), 400-meter walking test [6, 7].

Reference values for physical ability tests are as follows:

- 4-meter walking speed < 6 seconds is considered normal;
- SPPB test ≥ 10 points defines the well-fit physical form;
- TUG test < 10 seconds is considered normal;
- 400-meter walking test < 6 minutes is considered normal.

Muscle quality may be evaluated using the phase angle measured using BIA [6, 7].

The ultrasound method is recommended to measure the muscle amount and quality [6, 7].

The search for laboratory biomarkers of the muscle mass loss is considered perspective, requiring further studies.

Testing ammonia levels in patients with NAFLD and concomitant sarcopenia is important, as sufficient scientific data demonstrate that hyperammoniemia is an important factor of the impaired contractile skeletal muscle function [15]. Multiple studies have demonstrated that ammonia synthesis in hepatocytes is the main route of ammonia neutralisation. NAFLD with the impaired hepatocyte function promoting intestinal dysbiosis leads to the decreased tolerance to physical loads and hyperammoniemia. With that, skeletal muscles become the main organ accumulating ammonia, which impairs the contractile response of skeletal muscles. Leptin and other adipokines from the adipose tissue enhance the muscle catabolism and progressive hepatic fibrosis, which also makes the analysis of adipokines important when studying patients with sarcopenia and NAFLD [16] (Figure 1).

Increased blood cortisol levels may leads to insulin resistance (IR), metabolic syndrome (MS), increased levels of specific cytokines, and obesity. Consequently, cortisol may be a potential biomarker of sarcopenia and NAFLD.

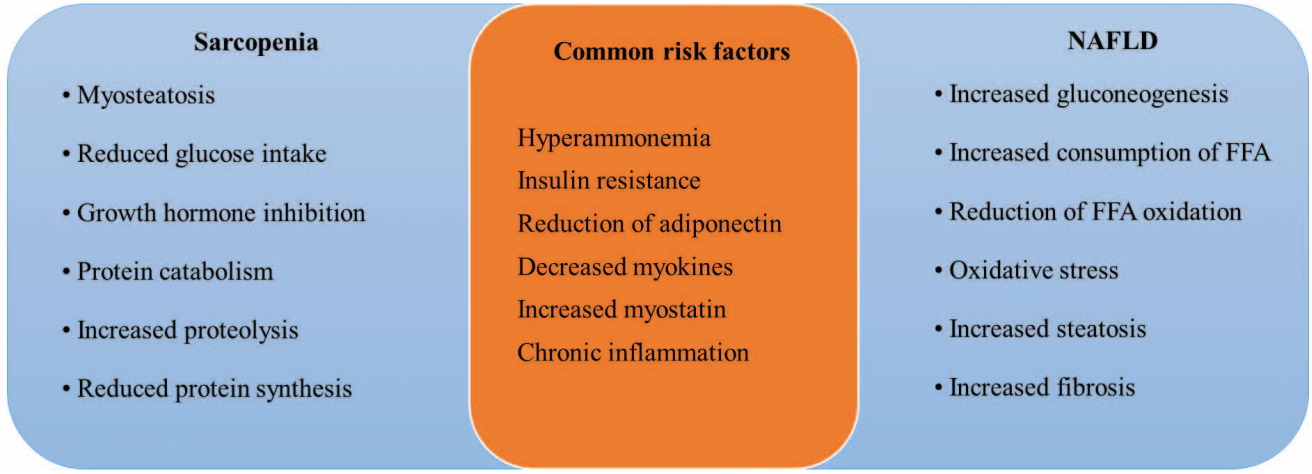

Figure. *Interaction between pathogenesis of sarcopenia and NAFLD.*
List of abbreviations: FFA — free fatty acids

Sufficiently significant data from studies demonstrate that skeletal muscles, liver, and adipose tissue exhibit variable activity (endocrine, autocrine, and paracrine) [17]. Secretion of cytokines and other signal molecules forms the basis of molecular crossover interactions in the „muscle-liver-adipose tissue" system, with cortisol acting as a key modulator. Chronic inflammation is considered a result of increased plasma levels of pro-inflammatory mediators, e.g. tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) [18]. Other circulating agents, i.e. TNF-like weak apoptosis inducer (TWEAK), IL-18, insulin-like growth factor 1 (IGF1), insulin, leptin, adiponectin, have been confirmed to be associated with sarcopenia and NAFLD — thus, they can be considered potential markers [19, 20]. Besides, the developing oxidative stress and accumulation of reactive oxygen species (ROS), trending to increase with age, cause severe lesions of the skeletal muscle cells, also participating in the NAFLD pathogenesis [19, 20]. Thus, testing for lipid peroxidation (LPO) and antioxidant protection (AOP) system may be important in patients with NAFLD and sarcopenia.

Actual issues of NAFLD and sarcopenia prevention and treatment

Non-drug aspects of NAFLD and sarcopenia treatment

According to modern national and European guidelines, a reconvalescent lifestyle is

feasible, which presumes a healthy diet, sufficient physical activity, body weight normalisation as a basis for the treatment and prevention of NAFLD, IR; this is especially important both for sarcopenia and „sarcopenic obesity" [3, 6, 7, 17, 21–24]. Guideline preferences focus on the Mediterranean diet, specifically counting the calories, with the optimal balance of vegetables, fruits, seafood, monounsaturated fatty acids, ω-3,6,9-polyunsaturated fatty acids, various plant fibers, and products with a low glycemic index. The diet also presumes rational limitation of sweet beverages and simple carbohydrates, increased amounts of insoluble dietary fibers to decrease the risk of hepatic steatosis and the risk of associated metabolic disorders [3, 21–24].

According to many studies, the efficacy of the Mediterranean diet has been demonstrated in the treatment of NAFLD with a sufficiently high degree of significance regarding the improvement in steatosis, inflammatory processes, and insulin resistance. However, its effects on the hepatic fibrosis degree remains less significant [21–24]. In overweight patients, World Health Organization (WHO) and European Liver Association guidelines define the necessity of the steady smooth weight decrease (no more than 0.5–1.0 kg per week). Many authors underline that weight loss > 5 % may decrease the hepatic liver amount, 7–10 % — may decrease the inflammation, and > 10 % — may decrease fibrosis. Quick weight loss is undoubtedly dangerous regarding the steatohepatitis and fibrosis progression, as well as a more significant LPO/AOP imbalance, which can lead to the deteriorated condition in sarcopenia [22–24].

Patients with NAFLD, but without obesity are recommended to lose weight moderately (3–5 %) to achieve the disease remission [24].

Physical activity is recommended in the form of predominantly aerobic, to the lesser extent — strength training both in NAFLD and sarcopenia, accounting for the individual approach. Physical activities should correlate to the NAFLD and sarcopenia stage, as well as with concomitant diseases. Exercises should be selected strictly individually.26 With that, one should account for the patient preferences, which will increase his/her compliance. It has been detected that physical exercises in the adequate scope arranged constantly lead to the improved histological signs in NASH even if the body weight has not decreased significantly. Besides, the physical activity

option proposed above promotes the decreased serum cholesterol levels**5** [21]. This is specifically important in the treatment of sarcopenic obesity.

It has been shown that physical activity is an efficient method of maintaining the normal function of muscles and the cardiovascular system. The combination of aerobic and strength exercises may be considered as a therapeutic strategy in patients with primary or secondary sarcopenia [7].

As physical abilities vary in different patients, it is important to use the personalised approach to each patient when selecting the physical exercise program. Electrical myostimulation and vibration have been proposed in patients with sarcopenia on a prolonged bed regimen as new therapeutic interventions. However, additional studies are required to determine their efficacy.

It has been established that the use of several diets (Mediterranean, Scandinavian) are associated with the improved physical functions and the decreased sarcopenia risk. One should thoroughly and individually weigh the risks and advantages before administering diets to decrease the weight in elderly persons with obesity due to the risk of muscle mass loss in them. When losing weight, to achieve the target loss of approximately 5–10 % from the primary body weight within 6 months, using protein in the amount at least 1.0 g/kg of body weight and fluid in the amount of at least 1.6–2.0 L of water daily will be required. Dietary regulations in patients with sarcopenia should provide them with sufficient calories, guarantee the adequate consumption of nutrients according to the individual features (age, sex, treatment, physical activity level) [25–28].

Drug aspects of NAFLD and sarcopenia treatment

No drugs have been currently approved for the treatment of NAFLD and sarcopenia [3, 6, 7, 21–23]. Therapeutic approaches for the combination of NAFLD and sarcopenia may be implemented via the increased tissue insulin sensitivity and decreased hepatic lesions.

Omega-3,6,9-polyunsaturated fatty acids are proposed for use in the treatment of NAFLD and sarcopenia. Currently these drugs are considered the first line in the treatment of hypertriglyceridemia in patients with NASH [22].

It is feasible to use statins to decrease cardiovascular risks in patients with NAFLD.

Statins decrease levels of cytolytic liver enzymes, decrease inflammation and steatosis in patients with NAFLD. Rosuvastatin is somewhat better to recommend, as it is the safest and most efficient. Antiangiogenic and antineoplastic effects define the preventive direction of lipophilic statin effects [29, 30].

Potential statin properties promoting sarcopenia may be associated with mechanisms mediated by infammatory processes, apoptosis, disorders in the ubiquitine-proteasome system, as well as with changes in insulin-like growth factor 1 and myostatin levels [31].

Increased triglyceride (TG) levels in blood may be adjusted with fibrates. Fenofibrate is an optimal representative of this drug group [23, 28]. This drug increases the insulin sensitivity of tissue receptors. In turn, this prevents lipid accumulation in muscles and the liver.

Fenofibrate as monotherapy or in combination with statins improves the atherogenic lipid serum profile, significantly decreasing TG levels, while increasing high-density lipoprotein (HDL) levels. Besides, it exhibits anti-inflammatory and antithrombotic effects, simultaneously improving the endothelial function, especially in patients with MS and DM2 [32].

Muscular complications, i.e. myalgia, myopathy, and rhabdomyolysis with increased creatine phosphokinase (CPK) levels, are considered the most common effects of statins and fibrates; they become the main causes for the adjustment of initial treatment with these drugs in sarcopenia [33, 34].

It is interesting to note that the daily tocopherol dose (vitamin E) dose of 800 mg positively affects histological parameters in NAFLD, including the improvement in balloon dystrophy, steatosis, and inflammatory processes. However, no improvement is detected in the hepatic fibrosis setting. At the same time, one should pay attention to the potential procarcinogenic tocopherol effects in doses ≥ 800 mg/day, which were detected in the context of prostatic cancer. The dose of 400 mg/day is considered optimal [3, 35]. The association between this drug and the muscle tissue condition is being actively analysed.

Tocopherol in combination with ursodeoxycholic acid (UDCA) is used in the treatment of patients with NAFLD. The efficacy of this combination is associated with decreased serum transaminase levels, decreased steatosis and hepatic inflammatory processes in NASH. The concomitant use of these drugs promotes the decreased hepatocyte apoptosis and improved hepatic histology. Besides, blood adiponectin levels restore, which is associated with metabolic and cytoprotective effects [36].

UDCA demonstrates a wide spectrum of pleiotropic effects, which promote its efficacy in the treatment of NAFLD. In particular, it possesses antioxidant, antifibrotic, and cytoprotective properties in hepatocytes. Besides, UDCA normalises apoptotic processes: if the apoptosis level was high, it promotes the decrease, though it can activate the process if apoptosis was lacking. This UDCA feature is the key for its anticarcinogenic effects. UDCA use also leads to the decreased aggressive effects of toxic bile acids on hepatocytes. When using UDCA at the NASH stage, functional hepatic parameters improve in patients with NAFLD. Besides, UDCA affects insulin resistance, which is one of the main pathogenetic mechanisms of NAFLD and metabolic syndrome.

UDCA therapy in patients with NAFLD significantly decreases lipotoxicity signs, steatosis, and even hepatic

fibrosis. UDCA promotes decreased insulin resistance, normalised lipid profile, and provides positive effects on metabolic processes [23, 36, 37].

Several effects (inhibition of serotonin, bradykinin, histamine inflammatory reactions; decreased vascular permeability; anti-kinin, antiproliferative effects) define the anti-inflammatory properties of glycyrrhizinic acid. Glycyrrhizinic acid inhibits protein kinase C, which blocks CD4+ leukocyte receptors, implementing pseudo-corticoid effects. Antioxidant properties of glycyrrhizinic acid are associated with its ability to block LPO processes. The mechanism of this inhibition includes 5-lipoxygenase phosphorylation [23]. Besides, glycyrrhizinic acid interacts with prostaglandin E2, a prooxidant. All these effects have significant effect in the treatment of NAFLD and sarcopenia.

Randomised controlled trials (RCTs) aimed at evaluating the efficacy of using cholecalciferol (vitamin D) orally for the treatment and prevention of sarcopenia demonstrated ambiguous results. Currently no sufficient evidence confirms its efficacy in the treatment of sarcopenia [38].

As of today, no drug product is approved for the treatment of sarcopenia, including testosterone. Currently the administration of testosterone is feasible only in patients with the established cause of hypogonadism, as it improves the muscle mass and strength in patients with hypogonadism [39, 40]. The addition of testosterone is efficient to improve the muscle mass and muscle strength only in elderly patients with variable hypogonadism degrees. However, the efficacy of testosterone concerning the physical ability is very low [40].

Besides, no positive estrogen effects have been demonstrated in the treatment of sarcopenia. It has been shown that estrogens do not significantly affect muscle mass and strength. Thus, estrogen benefit in females with sarcopenia is mild; with that, estrogen therapy is associated with a higher risk of breast cancer. This issue is an important limitation regarding estrogen therapy in patients with sarcopenia and NAFLD [40].

Dehydroepiandrosterone (DHEA) is synthesised in males and females, having the ability to increase testosterone levels. Only several small and relatively short RCTs analysed DHEA effects on sarcopenia [41], which proves that further studies are required.

Synthetic androgen receptor modulators (SARM) do not bond with corticosteroid and progesterone receptors, but are characterised by a high variability of regulatory androgenic receptor properties. This enables them to affect skeletal muscles without androgenic effects. However, additional studies are required. Currently no synthetic androgen modulator has been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [42].

Several studies have demonstrated that peripheral β2-receptor agonist, salbutamol, usually administrated in chronic obstructive pulmonary disease and asthma, improves the protein metabolism rate in skeletal muscles with a positive protein synthesis balance. Despite the potential therapeutic benefit in muscle atrophy, salbutamol or other highly selective β2-agonists have never been analysed in patients with sarcopenia [42].

Metformin and other prospective molecules, e.g. exerkines (interleukin-6, TNF-α, interleukin-15, fibroblast growth factor 21, irisin, apelin, etc.) or xenolytics (dazatinib, quercetine, ruxolitinib, etc.) are currently analysed in preclinical studies for the prevention of muscle mass, strength, and physical ability losses [43–45].

The intestinal microbiome affects the amino acid absorption [46]. This means that a microbiome may promote sarcopenia. In the future, after corresponding RCTs, the intestinal microbiome correction may become a new direction in the treatment of sarcopenia.

Probiotics and prebiotics may become new tools in the treatment of sarcopenia. The studies of bacterial quorum-sensitive peptides, e.g. iAM373, produced by E. faecalis have detected a potentially new sarcopenia inducer both in animals and humans [47], which requires additional analysis.

Thus, there is still very little evidence of efficient drug use for the treatment or prevention of sarcopenia. Unless current studies (large-scale Phase 3 RCTs, in particular) confirm the drug efficacy in the context of sarcopenia, lifestyle modification, including adequate physical exercises and quality food, will remain the sole recommendation for this indication.

Prophylactic aspects of NAFLD and sarcopenia evolution

Based on the data from randomised controlled trials (RCTs) concerning sarcopenia prevention, EWGSOP2 recommends the concept of maximum muscle mass increase in young persons, its preservation in the middle age, and minimising losses in the elderly [7]. Physical activities are considered as the basis for both primary and secondary sarcopenia prevention. In physical activities, the production of pro-inflammatory cytokines decreases, along with the increased synthesis of muscle protein, production of pro-inflammatory cytokines, and glucose consumption — that decreases the risk of NAFLD and sarcopenia progression. Physical exercises stimulate myocytes to produce myokines, enhancing muscle innervation and angiogenesis, satellite cell proliferation and differentiation. Regarding the bone tissue, physical exercises activate osteocytes to produce osteokines, promoting mitochondrial biogenesis and (indirectly) the muscle tissue growth.

The role of inflammatory cytokines and chemotactic proteins, e.g. monocytic chemotactic protein 1 (CX3CL1), in trained muscles presumes the attraction of immune cells and facilitation of their migration and infiltration into muscles; simultaneously, the product

of IL-10 and IL-1 receptor antagonist switches the pro-inflammatory reaction to anti-inflammatory [48]. This leads to decreased levels of several inflammatory cytokines participating in systemic inflammatory reactions [48, 49]. In addition to the localised release of the vascular endothelium growth factor (VEGF), the levels of associated extracellular matrix proteins, 61/CNN1 cysteine-rich angiogenic protein, and /CNN2 connective tissue growth factor along with IL-8 increase after exercises, and these molecules play an important role in skeletal angiogenesis, activating the endothelial cell proliferation, capillary tube organisation, and extracellular matrix remodeling. The production of angiopoietin-like protein 4 (ANGPTL4) in trained muscles enhances angiogenesis in skeletal muscles to an even larger extent, also increasing vascular permeability and lipid metabolism in muscles. These secretory angiogenic factors cause enhanced angiogenic effects in muscles, enabling a larger amount of blood to enter muscles with a more efficient delivery of nutrients into the muscle tissue — this is an important function in the prevention and delay of sarcopenia progression [49, 50].

Currently the issue of developing therapeutic & diagnostic algorithms for combined NAFLD and sarcopenia phenotypes is still not resolved. Undoubtedly, this topic will form the basis of prospective studies.

Conclusion

1. A clear association has been detected between NAFLD and sarcopenia. The prevalence of sarcopenia increases with NAFLD progression, and, vice versa, sarcopenia increases the risk of NASH and/or liver fibrosis in patients with NAFLD, affecting the mortality in LC. Physicians should assess the association between sarcopenia and NAFLD. Sarcopenia may be a potentially treatable condition. Specific therapeutic recommendations have still not been defined for patients with sarcopenia and NAFLD. No algorithm exists for the management of such patients.

2. It is feasible to actively screen patients with NAFLD for sarcopenia, assessing their muscle strength, force, and function.

3. The multidisciplinary approach for patients with NAFLD and sarcopenia should include the participation of not only a gastroenterologist, endocrinologist, cardiologist, and gerontologist, but also a physical therapist, dietician, and nutrition specialist. The treatment efficacy may require an individual approach, combination of drug and non-drug interventions accounting for the NAFLD and sarcopenia staging, as well as concomitant diseases and possible complications. The alertness concerning concomitant sarcopenia in patients with NAFLD is important to prevent the disease progression and to decrease the risk of remote negative results when selecting the optimal time for the start of therapeutic interventions.

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Стаценко М.Е.: создание концепции и дизайна работы, окончательном утверждение для публикации рукописи, проверка критически важного интеллектуального содержания, автор согласен быть ответственным за все аспекты работы

Мязин Р.Г.: поиск информации, анализ и обобщение данных литературы; написание статьи

Устинова М.Н.: создание концепции и дизайна работы, поиск информации, анализ и обобщение данных литературы, сбор данных литературы

Тумаренко А.В.: сбор данных; анализ и обобщение данных литературы; написание статьи, подготовка статьи к публикации

Author contribution:

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

Statsenko M.E.: creation of the concept and design of the article, final approval for publication, verification of critical intellectual content, the responsible author

Myazin R.G.: the information search, analysis and synthesis of literature data

Ustinova M.N.: creation of the concept and design of the article, information search, collection of literature data

Tumarenko A.V.: literature data collection; analysis and synthesis of literature data preparing the article for publication.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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СОДЕРЖАНИЕ МОЛЕКУЛ МЕЖКЛЕТОЧНОЙ АДГЕЗИИ У ПАЦИЕНТОВ С COVID-19-АССОЦИИРОВАННЫМ ПОРАЖЕНИЕМ ЛЕГКИХ

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The Content of Intercellular Adhesion Molecules in Patients With COVID-19-Associated Lung Disease

Резюме

Цель. Оценить содержание молекул межклеточной адгезии: ICAM-1, ICAM-2, ICAM-3, NCAM, VCAM-1, PECAM-1, E-sel, P-sel, EpCAM, L-sel у пациентов с COVID-19-ассоциированным поражением легких и выявить наличие взаимосвязи между их концентрацией и тяжестью течения процесса. **Материалы и методы.** В исследование были включены 200 пациентов после перенесенного COVID-19-ассоциированного поражения легких через 1 месяц после выписки из моностационаров г. Читы. Исследуемые были разделены на группы по 50 человек, в зависимости от степени поражения легких по результатам проведения компьютерной томографии: 1-я группа (КТ-1), 2-я группа (КТ-2), 3-я группа (КТ-3), 4-я группа (КТ-4). В группу контроля были включены 56 относительно здоровых лиц, не болевших ранее коронавирусной инфекцией и другими острыми респираторными заболеваниями за последние 3 месяца. Все исследуемые группы были сопоставимы по полу и возрасту. Содержание молекул межклеточной адгезии в сыворотки крови определяли методом иммунохимического анализа. **Результаты.** В результате проведенного исследования было выявлено повышенное содержание молекул межклеточной адгезии (ММА) (ICAM-1, ICAM-2, ICAM-3, NCAM, VCAM-1, PECAM-1, E-sel, P-sel, EpCAM, L-sel) у исследуемых групп больных с COVID-19-ассоциированным поражением легких в сравнении с группой контроля. Были обнаружены различия между группами пациентов с разным уровнем поражения легких по данным КТ, при исследовании некоторых молекул межклеточной адгезии. **Заключение.** По итогам проведенной работы выявлено, что после перенесенной коронавирусной инфекции, осложненной поражением легких, в крови наблюдается повышение концентрации молекул межклеточной адгезии — представителей всех исследуемых суперсемейств. Увеличение уровней молекул межклеточной адгезии у исследуемых пациентов отражает наличие эндотелиоза и коррелирует с тяжестью поражения легочной ткани, в том числе и в период реконвалесценции.

Ключевые слова: *COVID-19-ассоциированное поражение легких, молекулы межклеточной адгезии, ICAM-1, ICAM-2, ICAM-3, NCAM, VCAM-1, PECAM-1, E-sel, P-sel, EpCAM, L-sel*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

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Abstract

Objective. To evaluate the content of intercellular adhesion molecules: ICAM-1, ICAM-2, ICAM-3, NCAM, VCAM-1, PECAM-1, E-sel, P-sel, EpCAM, L-sel in patients with COVID-19-associated lung damage and to identify the relationship between their concentration and severity the flow of the process. **Materials and methods.** The study included 200 patients after suffering COVID-19-associated lung damage 1 month after discharge from Chita monostationals. The subjects were divided into groups of 50 people, depending on the degree of lung damage according to the results of computed tomography: Group 1 (CT-1), median age was 51.5 [50.5; 54.8]; Group 2 (CT-2), median age 57.0 [53.1; 57.0]; group 3 (CT-3), median age 52.5 [51.9; 55.0]; 4th group (CT-4), median 55.0 [53.2; 56.4]. The control group included 56 relatively healthy individuals who had not previously had coronavirus infection and other acute respiratory diseases in the last 3 months, the median age was 55.0 [51.1; 55.0]. All the study groups were comparable in gender and age. The content of intercellular adhesion molecules in blood serum was determined by immunochemical analysis. **Results.** The study revealed an increased content of intercellular adhesion molecules (MMA) (ICAM-1, ICAM-2, ICAM-3, NCAM, VCAM-1, PECAM-1, E-sel, P-sel, EpCAM, L-sel) in the studied groups of patients with COVID-19-associated lung damage in comparison with the control group. Differences were found between groups of patients with different levels of lung damage according to CT data, when examining some intercellular adhesion molecules. **Conclusion.** According to the results of the work carried out, it was revealed that after a coronavirus infection complicated by lung damage, an increase in the concentration of intercellular adhesion molecules in the blood is observed — representatives of all the studied superfamilies. An increase in the levels of intercellular adhesion molecules in the studied patients reflects the presence of endotheliosis and correlates with the severity of lung tissue damage, including during the period of convalescence.

Key words: *COVID-19-associated lung damage, intercellular adhesion molecules, ICAM-1, ICAM-2, ICAM-3, NCAM, VCAM-1, PECAM-1, E-sel, P-sel, EpCAM, L-sel*

Conflict of interests

The authors declare no conflict of interests

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Conformity with the principles of ethics

The study was approved by the local Ethics Committee of the Chita State Medical Academy of the Ministry of Health of the Russian Federation (extract from Protocol No. 105. December 2, 2020). Informed consent was obtained from all subjects who participated in the study. Written informed consent was also obtained from the patients for the publication of this article.

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IAM — intercellular adhesion molecule, EC — endothelial cells, COVID-19 — novel coronavirus infection

Introduction

Intercellular adhesion molecules (IAM) are cell surface proteins, which participate in binding cells to one another and to the extracellular matrix. They are essential components in maintaining the tissue structure and functions. Also, IAMs participate in cell growth mechanism, contact cell inhibition and apoptosis, endothelial cell (EC) activation at the inflammation site, white blood cell migration from the vascular bed to adjacent tissues, pathogen and toxin eradication, vessel sequestration and remodelling, reparation and hemostasis mechanisms [1]. In the physiologically normal state, endotheliocytes do not express IAMs. Under the influence of damaging factors, IAM concentration on their surface increases, and oxidised lipids and lipoproteins accumulate in larger amounts in the subendothelial area [2]. Excessive, uncontrolled EC activation causes formation of small blood clots, higher vascular permeability, tissue and cell hypoxia, and results in inflammation [1, 3].

In terms of structural similarity, IAMs are stratified into five 5 superfamilies [4]:

1. Integrins (CD29 (β_1), CD18 (β_2), CD61 (β_3), CD49 (β_7), etc.) are hetero-dimeric molecules, which function both as cell substrate and intercellular adhesive receptors.
2. Adhesive receptors from immunoglobulin superfamily (PECAM-1, NCAM, ICAM-1, ICAM-2, ICAM-3, etc.) participate in intercellular adhesion.
3. Selectins (E-, P-, L-selectins) are adhesive receptors, the lectin-like domain of which ensures adhesion of white blood cells to endothelial cells.
4. Cadherins (E-, P-, N-, R-, VE-cadherins) are calcium-dependant adhesion proteins, which ensure contacts between endothelial cells.
5. Homing receptors or addressins (MAdCAM-1, mucosaladressin cellular adhesion molecule-1)), CD34, GlyCAM-1) are molecules, which ensure lymphocyte release to the lymphoid tissue.

Another IAM has been found recently, which is not included in any of the above molecule classes: EPCAM (epithelial cell adhesion molecule, CD326) — a membrane protein, encoded by a same-name gene, which participates in the intercellular adhesion in epithelium, signal transmission to the cell nucleus, cell migration, cell proliferation and differentiation, as well as dissemination of tumours [5].

There are membrane-dependent and soluble IAMs, their main function being regulation of white blood cell migration from the blood flow via endothelium to the cell damage area [2].

The process of white blood cell migration from the vessel bed via endothelium runs in several steps, and IAMs participate in each and every of them. The process of "border standing", as a result of which white blood cells stay at the borderline of the vessel bed, is facilitated by P-selectin. White blood cell activation (early adhesion) is also promoted by selectins (P- and E-selectins). "Other white blood cell adhesion" is ensured by ICAM-1 and ICAM-2 as well as by leucocyte integrins (LFA-1 and Mac 1). Transendothelial white blood cell migration is facilitated by the same integrins and ICAM-1, VCAM-1, PECAM-1 [4].

Given that IAMs participate in immune response and inflammation progression, the academic interest lies in the research of their expression in various contagious pathologies, including COVID-19-assoiciated lung damage.

Study Objective

To study the levels of intercellular adhesion molecules (ICAM-1, ICAM-2, ICAM-3, NCAM, VCAM-1, PECAM-1, E-sel, P-sel, EpCAM, L-sel) in patients with COVID-19-assoiciated lung damage and identify the correlation between their concentration and condition severity.

Materials and Methods

The study included 200 patients, who previously had COVID-19-assoiciated lung damage and were discharged from specialised inpatient clinics in Chita a month before enrolment. All patients were divided into groups of 50 people, depending on the degree of lung damage as seen on CT scans: group 1 (CT1) — median age was 51.5 [50.5; 54.8]; group 2 (CT2) — median age was 57.0 [53.1; 57.0]; group 3 (CT3) — median age was 52.5 [51.9; 55.0]; and group 4 (CT4) — median age was 55.0 [53.2; 56.4]. The study included patients with confirmed COVID-19; they had a positive polymerase chain reaction test for SARS-CoV-2 RNA. Exclusion criteria were: systemic diseases; lymphoproliferative and myeloproliferative disorders requiring immunosuppressive therapy; pregnancy, HIV infection, chronic alcoholism. The control group included 56 healthy volunteers without a history of coronavirus infection and other respiratory diseases within the past three months; median age was 55.0 [51.1; 55.0]. All study groups were comparable in sex and age composition. The levels of serum intercellular adhesion molecules were measured using immunochemical assay. Statistical processing of study results was performed with IBM SPSS Statistics Version 25.0 (licence No. Z125-3301-14, IBM, USA) [6,7].

Results and Discussion

ICAM-1 (CD54) is an integral membrane protein, included in the immunoglobulin superfamily. In the physiologically normal state, its expression by endothelial cells is close to zero. Its expression rises under the influence of free radicals, complement components, nitrogen oxide, lipopolyssacharides, pro-inflammatory cytokines (IL-1, 6, 8; TNF-α, etc.), histamine, leukotriene and other mediators [8–9]. Also, this IAM is expressed by lymphocytes, monocytes, bronchoalveolar epithelial cells, and the expression increases within 6–8 hours after stimulation and persists for 48 hours [2]. The role of ICAM-1 as a marker of diseases, including inflammatory conditions, has been proven by a number of pathological reactions. In allergic inflammation of airways, ICAM-1 promotes the development of nasal allergies. Higher IAM (sICAM-1) levels were found in HIV-1 carriers. According to G.P. Downey and L. Fialkow (1995) [10], plasma sICAM-1 levels are a prediction criterion; a value of over 1,000 ng/mL is a sign of a high probability of death [11]. A study of A/H1N1/09 pneumonias showed that sICAM-1 levels in patients with various degrees of disease severity had multidirectional fluctuations: they were higher in more severe cases and lower in mild pneumonias; in a vast majority of patients with the highest sICAM-1 levels, pneumonia was associated with acute lung damage [12].

An analysis of ICAM-1 concentrations in the study groups showed its higher levels in patients with COVID-19-associated lung damage vs. controls. Group 1 (CT-1) — 1.2 times higher [1.4; 2.03] ($p < 0.001$), group 2 (CT-2) — 1.8 times higher [2.2; 2.2] ($p < 0.001$), group 3 (CT-3) — 1.9 times higher [1.1; 2.2] ($p < 0.001$), group 4 (CT-4) — 2.01 times higher [1.3; 2.8] ($p < 0.001$) (Table 1). Also, lower ICAM-1 concentrations were observed in patients with mild COVID-19-associated lung damage (CT-1) vs. groups CT-2, CT-3, CT-4: 1.4, 1.5 and 1.6 times higher, respectively ($p < 0.001$) (Figure 1).

ICAM-2, another representative of the immunoglobulin superfamily, is found on the cell membrane surface, mostly of hemopoietic cells [2, 13]. Its expression on dormant lymphocytes is higher than that of ICAM-1, whereas the synthesis of these molecules on monocytes is approximately the same. Similarly to ICAM-1, its receptor is integrin LFA-1. Since unlike ICAM-1, ICAM-2 is found on dormant ECs, it is likely to participate in recirculation of LFA-1-positive lymphocytes. Also, ICAM-2 is essential for initiation of T-cell adhesion to antigen-presenting cells. For now, it is assumed that an additional function of ICAM-2 is ICAM-1-independent lysis of various target cells [2, 14].

In this study, ICAM-2 levels were higher in patients in CT-1, CT-3, and CT-4 groups vs. healthy subjects (Table 1): CT-1 — 1.2 times [1.01; 1.3] ($p = 0.025$),

CT-3 — 1.4 times [1.2; 1.7] ($p < 0.001$), CT-4 — 1.6 times [3.5; 1.04] ($p < 0.001$). There are also differences in the concentration of this IAM between the group of mild lung damage (CT-1, CT-2) and the group of severe COVID-19-associated lung damage (CT-3, CT-4) (Figure 2).

ICAM-3 is an integral membrane protein with high homology to ICAM-1 and ICAM-2 in the extracellular region; it is expressed on dormant lymphocytes, monocytes and neutrophils. ICAM-1, ICAM-2, and ICAM-3 are a ligand for LFA-1, which impacts its activity. Unlike ICAM-1 and ICAM-2, ICAM-3 is not present on

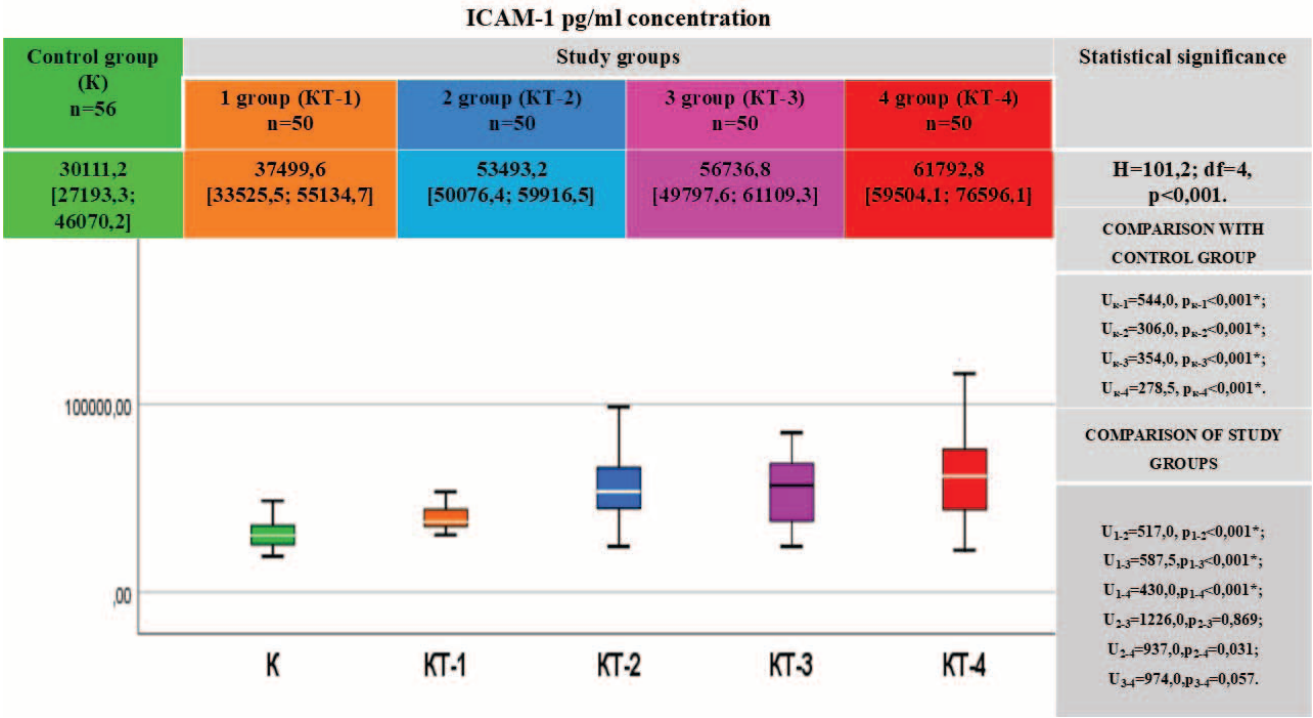

Figure 1. *The concentration of ICAM-1 intercellular adhesion molecules in the blood of patients in the studied groups*

Note: statistical significance of differences between: p_{c-1} — control group and group 1; p_{c-2} — control group and group 2; p_{c-3} — control group and group 3; p_{c-4} — control group and group 4; p_{1-2} — between groups 1 and 2 of patients; p_{1-3} — between 1 and 3 groups of patients; p_{1-4} — between 1 and 4 groups of patients; p_{2-3}– between 2 and 3 groups of patients; p_{2-4} — between 2 and 4 groups of patients; p_{3-4} — between 3 and 4 groups of patients.

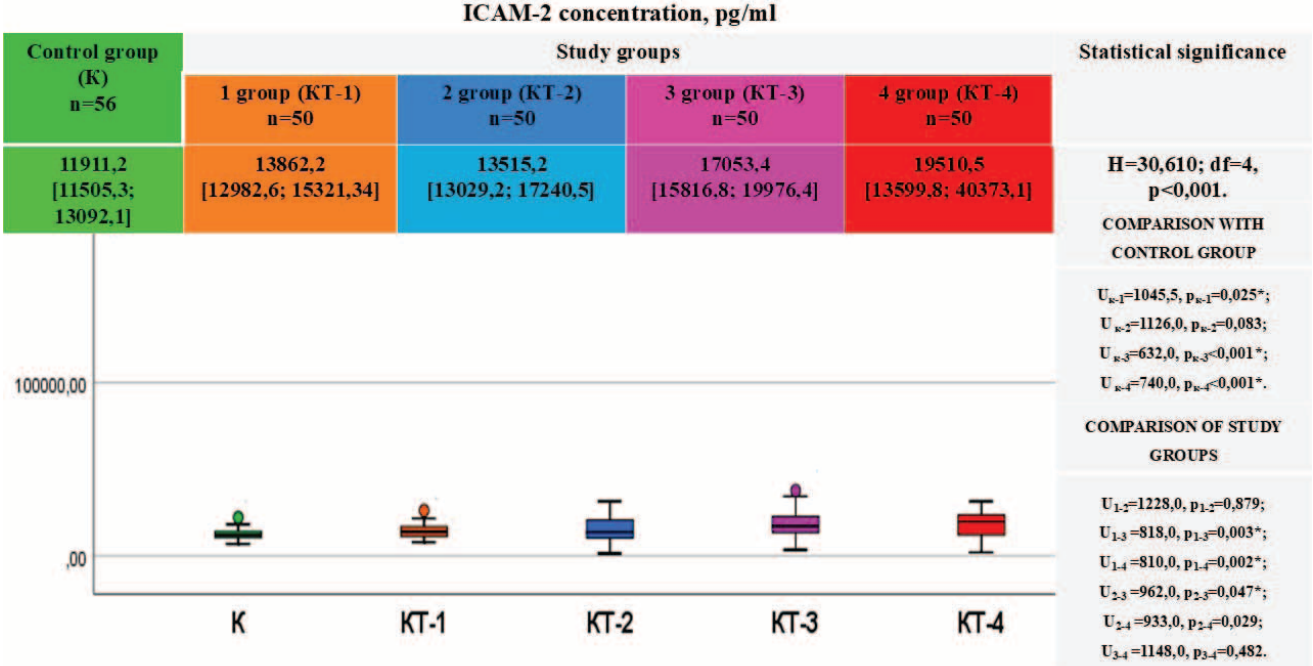

Figure 2. *The concentration of ICAM-2 intercellular adhesion molecules in the blood of patients in the studied groups*

Note: see figure 1

endothelium, but its expression is better on monocytes and dormant lymphocytes vs. other LFA-1 ligands [2, 16]. An analysis of the available information shows that ICAM-3 has a crucial role to play in immune response initiation. It has also been found that this IAM participates in regulation of LFA-1/ICAM-1-dependent intercellular white blood cell interaction. sICAM-3 levels increase in rheumatoid arthritis, systemic lupus erythematosus [1].

An analysis of serum ICAM-3 levels in patients demonstrated higher concentrations in patients with coronavirus infection vs. controls: group 1 (CT-1) — 1.9 times [1.6; 2.7] ($p < 0.001$), group 2 (CT-2) — 1.7 times [1.3; 2.3] ($p < 0.001$), group 3 (CT-3) — 1.4 times [1.1; 2.03] ($p < 0.001$), in group 4 (CT-4) — 1.4 times [1.1; 2.01] ($p < 0.001$). Comparison of patients with COVID-19-associated lung damage revealed statistically significant differences in ICAM-3 concentrations between patients with mild lung damage (CT-1) and patients with severe condition (CT-3, CT-4) ($p = 0.001$). Of note, a higher concentration of this IAM was observed in patients with smaller areas of pulmonary tissue damage caused by coronavirus infection (CT-1) as compared to patients in CT-3 and CT-4 groups: comparison of group 1 (CT-1) and group 3 (CT-3) — 1.4 times [1.03; 1.8] ($p = 0.001$); of group 1 (CT-1) and group 4 (CT-4) — 1.4 times [1.04; 1.8] ($p = 0.001$) (Figure 3).

NCAM (CD56), a neural cell adhesion molecule, is a homophilic binding glucoprotein, which is expressed on the surface of neurons, glia and skeletal muscles. Its expression was also found in the hematopoietic system; it is associated with natural killer cells, but is not limited to them. CD56 was found on other lymphocytes, including γδ T cells and activated CD8+ T cells, as well as dendritic cells. According to present knowledge, NCAM participates in cell adhesion, axon spread, synaptic plasticity, learning, and memory [2,17].

An analysis of this IAM in our patients showed its higher concentrations: group 1 (CT-1) vs. controls — 1.4 times [1.2; 1.6] ($p < 0.001$); group 2 (CT-2) — 1.6 times [1.9; 1.4] ($p < 0.001$); group 3 (CT-3) — 1.8 times [1.5; 2.01] ($p < 0.001$); group 4 (CT-4) — 2.2 times [1.9; 2.5] ($p < 0.001$). There is also difference in NCAM levels between patients with mild COVID-19-associated lung damage (CT-1) and other study groups (CT-2, CT-3, CT-4): 1.1 [1.1; 1.4]; 1.3 [1.1; 1.4]; 1.5 [1.4; 1.7] timely, respectively ($p < 0.001$) (Figure 4).

VCAM-1 is involved in leukocytic-endothelial interaction and expressed after cell stimulation by IL-1, TNF-α or an endotoxin. This IAM is a ligand to integrin VLA-4, found on lymphocytes, monocytes and eosinophils [2]. It participates in white cell adhesion outside vessels, thus ensuring interaction between lymphoblasts and stromal cells of the bone marrow and between B cells and dendritic cells in lymph node follicles. According to the literature, VCAM-1 possesses selective leukocytic adhesion, thus ensuring mononuclear cell accumulation during acute inflammation [18, 19]. Higher VCAM-1 levels were observed in various autoimmune conditions (multiple sclerosis, systemic sclerosis, systemic lupus erythematosus), infections (sepsis, meningitis, malaria) and other [1, 2, 18].

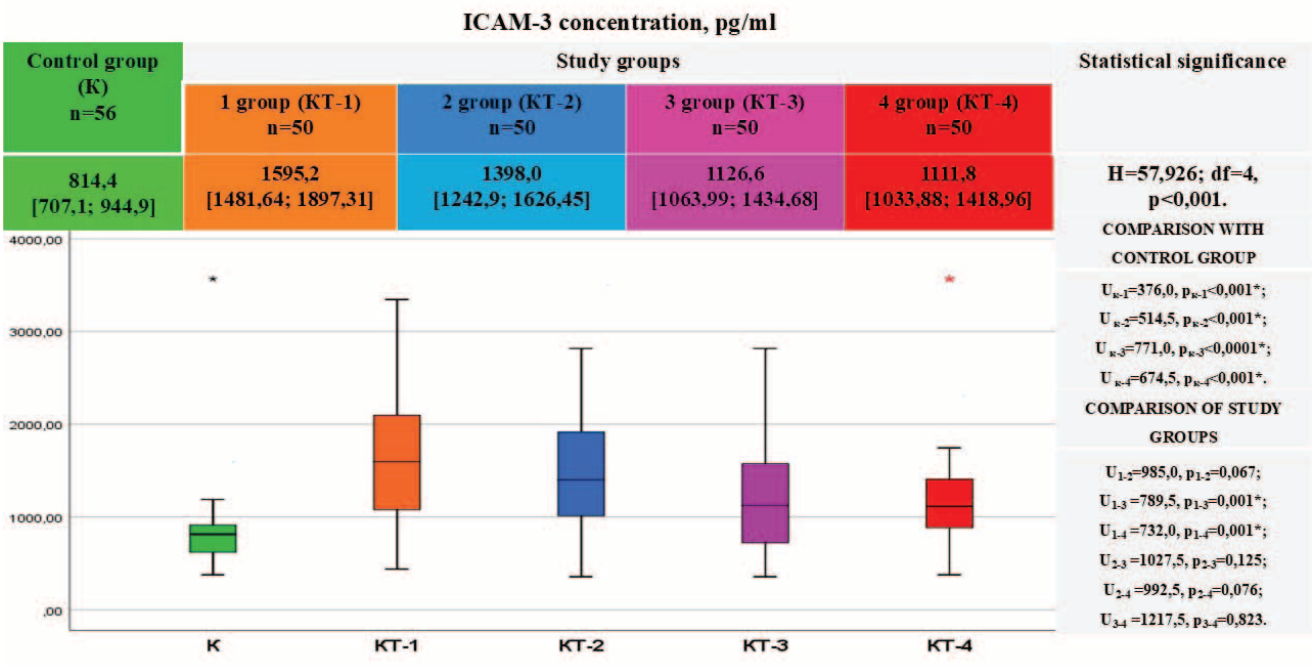

Figure 3. *The concentration of ICAM-3 intercellular adhesion molecules in the blood of patients in the studied groups*
Note: see figure 1

A study of concentrations of this molecule in post-coronavirus infection patients demonstrated higher levels in patients with COVID-19-associated lung damage (CT-2, CT-3, CT-4) vs. controls: 1.3, 1.7, 1.8 times, respectively ($p < 0.001$). Serum VCAM-1 levels were higher in patients with a more severe condition (Figure 5).

PECAM-1 (CD31) is a transmembrane glucoprotein; it is expressed mostly by vascular cells and is an immunohistochemical marker of blood vessel angiogenesis. CD31 was found on platelets, monocytes, neutrophils, and CD8+ T cells [2]. Previous studies confirm PECAM-1 involvement in inflammatory processes and interaction between white blood cells and ECs. It has also been established that, during white cell migration, they enter the inflammation site via intercellular junctions of vessel endotheliocytes under the influence of PECAM-1 [20, 21].

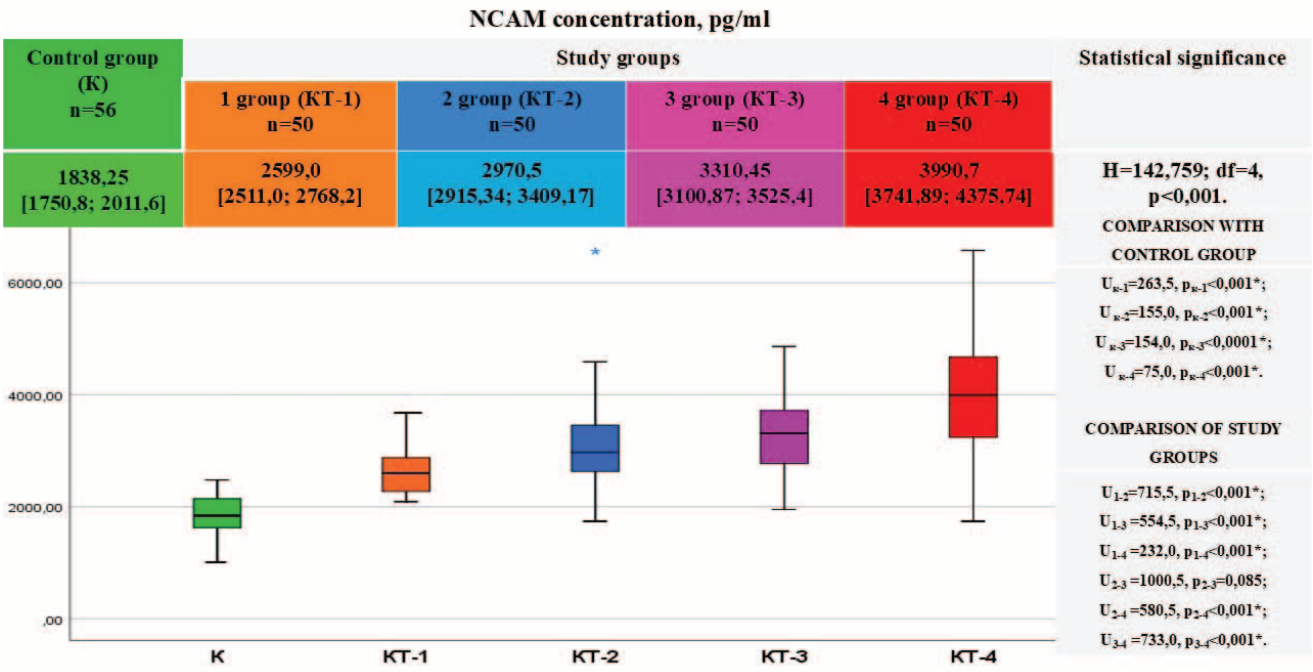

Figure 4. *The concentration of NCAM intercellular adhesion molecules in the blood of patients in the studied groups*
Note: see figure 1

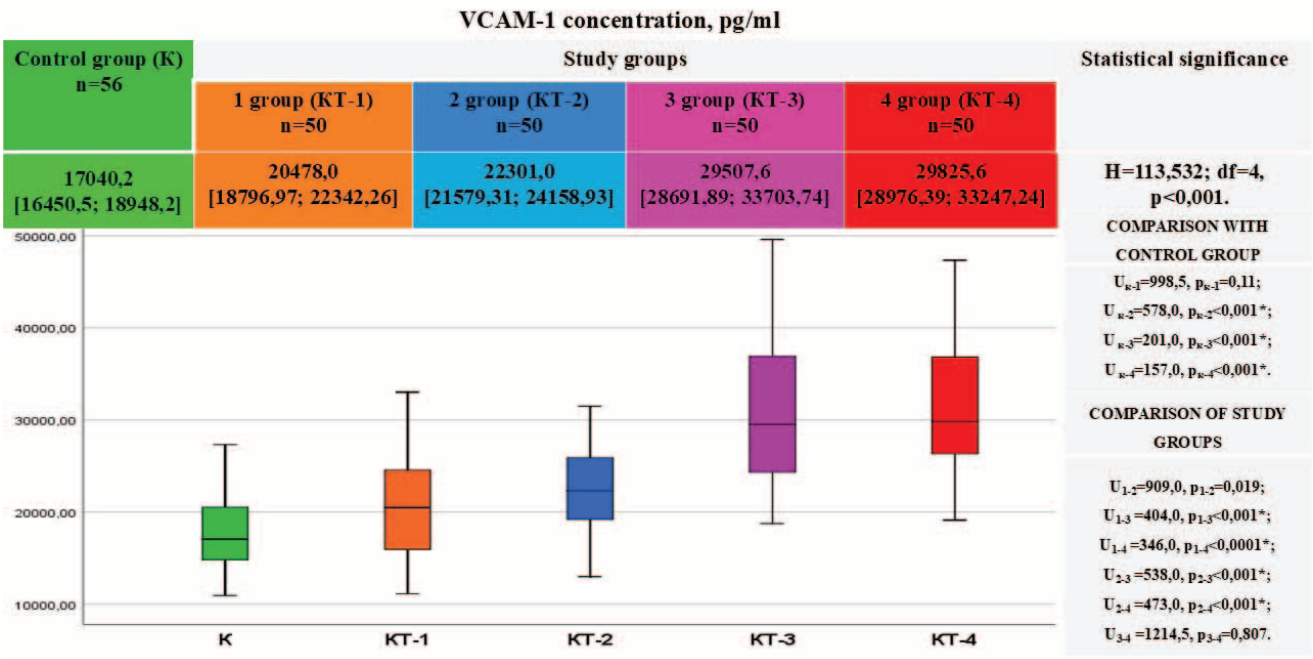

Figure 5. *The concentration of VCAM-1 intercellular adhesion molecules in the blood of patients in the studied groups*
Note: see figure 1

Studies of PECAM-1 showed its higher concentrations in patients vs. controls. Higher PECAM-1 levels were observed in patients with COVID-19-associated lung damage (CT-1) vs. controls: 1.4 times [1.2; 1.6] ($p < 0.001$), and 1.6 times [1.5; 1.9] vs. CT-2, CT-3 and CT-4 ($p < 0.001$) (Figure 6).

Selectins (cluster of differentiation 62, or CD62) are cell membrane glucoproteins, which ensure adhesion interactions between hematopoietic, tumour cells, white blood cells, platelets and endothelium. Cell adhesion has a crucial role to play in inflammatory, infectious, metastatic and immune processes, as well as in the ability of stem cells to identify their "niche" [22, 23]. Selectins are essential for rolling and adhesion of polymorphonucleocytes to endothelial wall, their migration to the intercellular matrix [24–26]. As we know, selectins are practically not expressed on membranes of non-activated cells. When endothelial cells, white blood cells and platelets are activated under specific conditions (changes in blood flow velocity, pH and temperature, impaired cell structure, exposure to biologically active molecules), their expression increases [22, 27]

E-selectin (E-sel) is expressed by endothelial cells when endothelium is damaged as well as in case of an inactive, long-lasting non-specific inflammation, promoting white blood attraction (chemotaxis) [24, 28, 29]. E-selectin is synthesised on endothelial cell membrane 4–6 hours after exposure to tumour necrosis factor α, interferon γ and interleukin-1. This selectin is involved in initiation of activated white cell adhesion to endothelial cells in the inflammation site [22, 30]. The highest selectin E concentrations can persist for 1–2 days. With lower levels, slow white cell rolling and inflammation severity drop. This molecule is involved in adhesion of endotheliocyte precursor, facilitating their migration and formation of capillaries. Introduction of an adenoviral vector of E-selectin promotes formation of capillaries and reduces severity of necrosis caused by ischaemia. Thus, E-selectin hyperexpression proves its involvement in adhesion of endothelial cell precursors and neoangiogenesis [22, 31, 32].

P-selectin (P-sel) is found in α-granules of platelets and secretory granules (Weibel-Palade bodies) of endothelial cells; they are involved in primary interaction between polymorphonuclear neutrophils and endothelial cells, particularly in the inflammation site. It has been proven that, when acting together with cytokines, it can regulate integrin synthesis. The highest concentration is observed 5–10 minutes after cell activation, and within half an hour/an hour P-selectin is detected on cell surfaces [22, 33]. According to G. V. Chaitanya et al., P-selectin expression can be regulated under the influence of nitrogen oxide [34, 35]. Besides, expression of this selectin on endothelial cell surface increases during hypoxia and reduces in hypoglycaemia [36].

L-selectin (L-sel) is involved in white cell migration to inflamed tissues; higher levels of L-selectin ligands initiate its expression. The important role of L-selectin is adhesion of circulating white blood cells to white blood cells adhering to a vessel wall, known as secondary binding. This selectin is constantly produced on white blood cells and quickly leaves the cell surface after its activation. It helps white cells to adhere to lymph node cells and activated endothelium [37, 38].

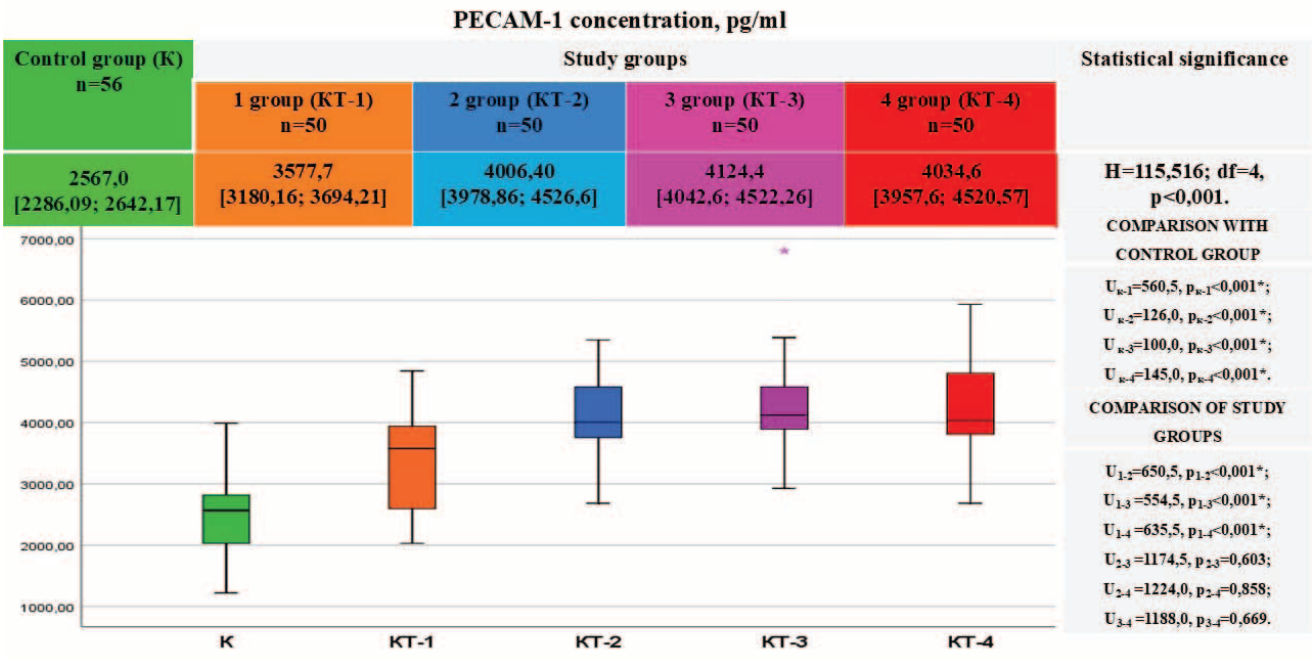

Figure 6. *The concentration of PECAM-1 intercellular adhesion molecules in the blood of patients in the studied groups*
Note: see figure 1

A study of E-selectin in patients after coronavirus infection showed higher concentrations in the study groups vs. controls: group 1 (CT-1) vs. controls — 1.5 times [2.4; 4.4] (p = 0.007); group 2 (CT-2) — 1.9 times [1.7; 4.8] (p < 0.001); group 3 (CT-3) — 1.8 times [1.5; 3.2] (p < 0.001), group 4 — (CT-4) — 1.7 times [1.6; 2.6] (p < 0.001) (Figure 7).

Analysis of P-selectin concentrations demonstrated similar results; its levels were higher in patients after COVID-19 as compared to controls. group 1 (CT-1) — 1.1 times [1.1; 1.9] (p = 0.002), group 2 (CT-2) — 1.7 times [1.4; 2.5] (p < 0.001), group 3 (CT-3) — 1.8 times [1.5; 2.5] (p < 0.001), in group 4 (CT-4) — 1.9 times [1.6; 2.8] (p < 0.001). Also, significantly higher levels of P-selectin were observed in patients with lung damage (CT-2, CT-3, CT-4) as compared to CT-1 patients: CT-2 vs. CT-1 — 1.5 times [1.01; 1.8] (p = 0.008), CT-1 and CT-3 — 1.6 times [1.02; 1.8] (p = 0.002), CT-4 and CT-1 — 1.7 times [1.1; 1.9] (p < 0.001) (Figure 8).

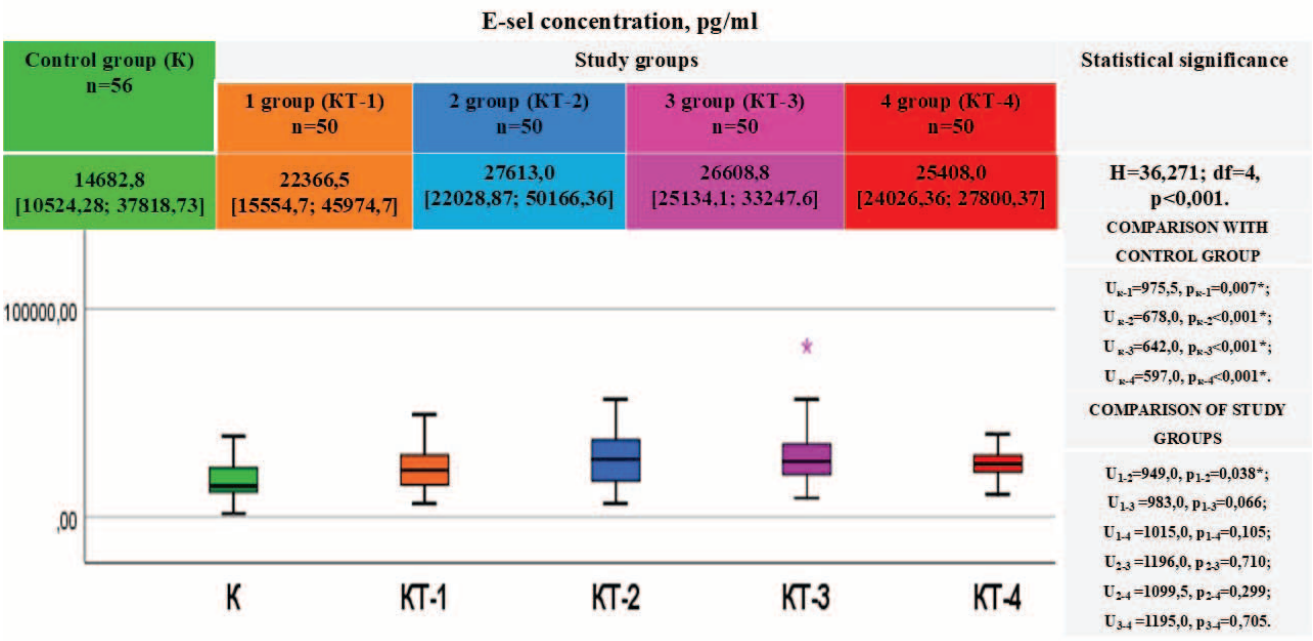

Figure 7. *The concentration of intercellular adhesion molecules of E-selectin in the blood of patients in the studied groups*
Note: see figure 1

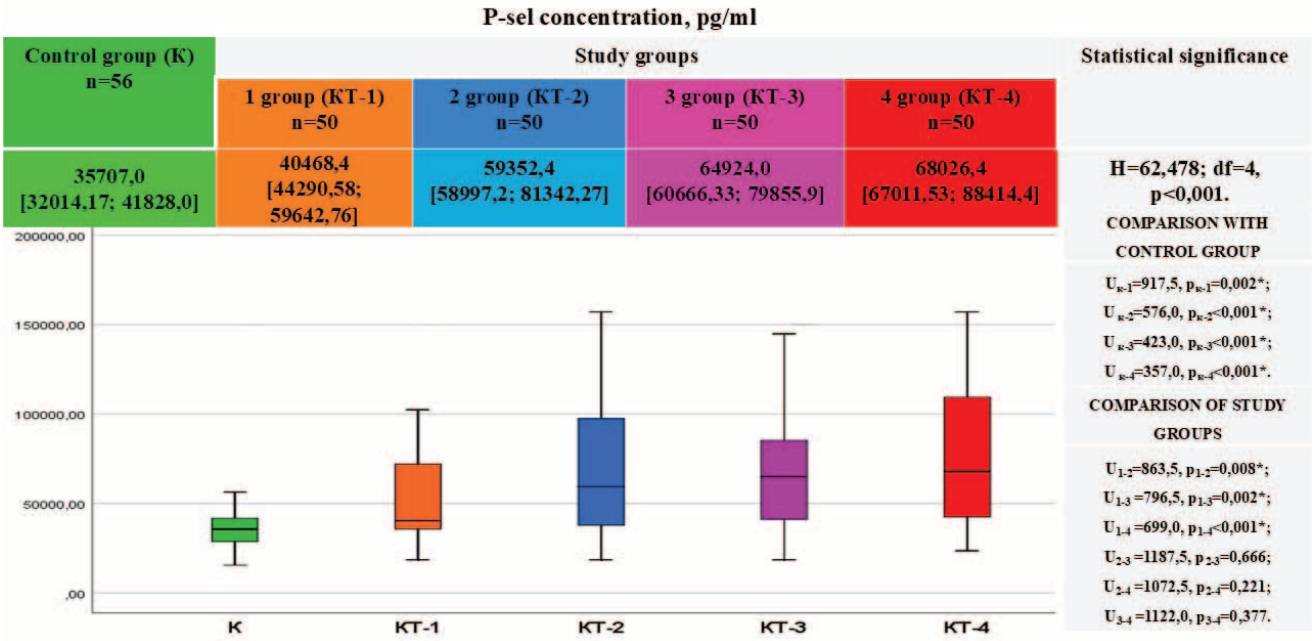

Figure 8. *The concentration of intercellular adhesion molecules of P-selectin in the blood of patients in the studied groups*
Note: see figure 1

An analysis of L-selectin levels in this study also demonstrated high concentrations in the study groups vs. controls: group 1 — 1.4 times [1.3; 1.7] ($p < 0.001$); group 2 — 1.2 times [1.1; 1.4] ($p < 0.001$); group 3 — 1.2 times [1.1; 1.5] ($p < 0.001$); group 4 — 1.2 times [1.4; 1.1] ($p = 0.001$). Excess concentrations of L-selectin were recorded in patients with CT-1 lung damage as compared to patients from CT-2, CT-3, CT-4 groups: CT-1 vs. CT-2 — 1.1 times [1.02; 1.4] ($p = 0.001$), CT-1 vs. CT-3 — 1.1 times [1.01; 1.3] ($p = 0.004$), CT-1 vs. CT-4 — 1.1 times [1.4; 1.05] ($p = 0.002$) (Figure 9).

EPCAM is a type I transmembrane glucoprotein; it plays an important role in cell adhesion and is expressed mainly in the large and small intestine, pancreas. Its intercellular binding is ensured by the extracellular domain of this protein; however, EPCAM-mediated intercellular contacts are relatively weak. EPCAM affects cadherin-mediated cell interaction by diminishing the association of the cadherin-catenin complex in cytoskeleton. Higher EPCAM expression lowers alpha catenin levels. Active proliferation in epithelial tissues is associated with increased EPCAM synthesis, whereas epithelial cell differentiation is associated with its decrease [39, 40]. This molecule possesses oncogenic potential: it can boost the activity of c-myc, e-fabp proteins, cyclins A and E and can be a marker of some cancer types due to specific expression only in epithelium and epithelial tumours [40].

A study of EPCAM showed its higher levels in patients with CT-1 lung damage as compared with controls: 1.6 times [1.2; 2] ($p < 0.001$). There are differences between study groups: mild lung damage (CT-1) and severe involvement (CT-3, CT-4) after the past coronavirus infection. EPCAM levels were 1.5 times higher when comparing CT-3 to CT-1 [1.01; 1.8] ($p = 0.007$) and 1.4 times higher when comparing CT-4 to CT-1 [1.03; 1.9] ($p = 0.005$) (Figure 10).

The available references evidence that COVID-19 patients have higher levels of soluble cell adhesion molecules, especially of ICAM-1 [41]. A retrospective study of COVID-19 patients in China showed that sICAM-1 concentrations in the blood increased with increasing disease severity. This parameter normalised during the recovery phase [42]. In patients with COVID-19 and cirrhosis, higher ICAM-1 levels were an independent predictor of death [43]. An analysis of blood ICAM-1 concentrations in COVID-19 patients 2 to 33 weeks after the diagnosis showed that ICAM-1 levels remained low two weeks after COVID-19 diagnosis and increased six-fold five weeks, and then normalised [42]. Similar data are available for ICAM-1 molecules in COVID-19 patients two weeks and five weeks after COVID-19 diagnosis: five weeks later, ICAM-1 levels increased six-fold vs. baseline values [42, 43].

In this study, plasma IAM levels were measured one month after discharge from a specialised hospital, and results are similar to the data presented by other authors, who studied the levels of endothelial dysfunction molecules at various stages after the past COVID-19 infection.

Following the study, a binary logistic analysis was performed in order to assess the influence (identification of independent predictive factors) of the test parameters on the probability of pulmonary fibrosis after COVID-19-associated lung damage. The model was based on the logistic regression method with sequential exclusion of the least significant factors. Selection and

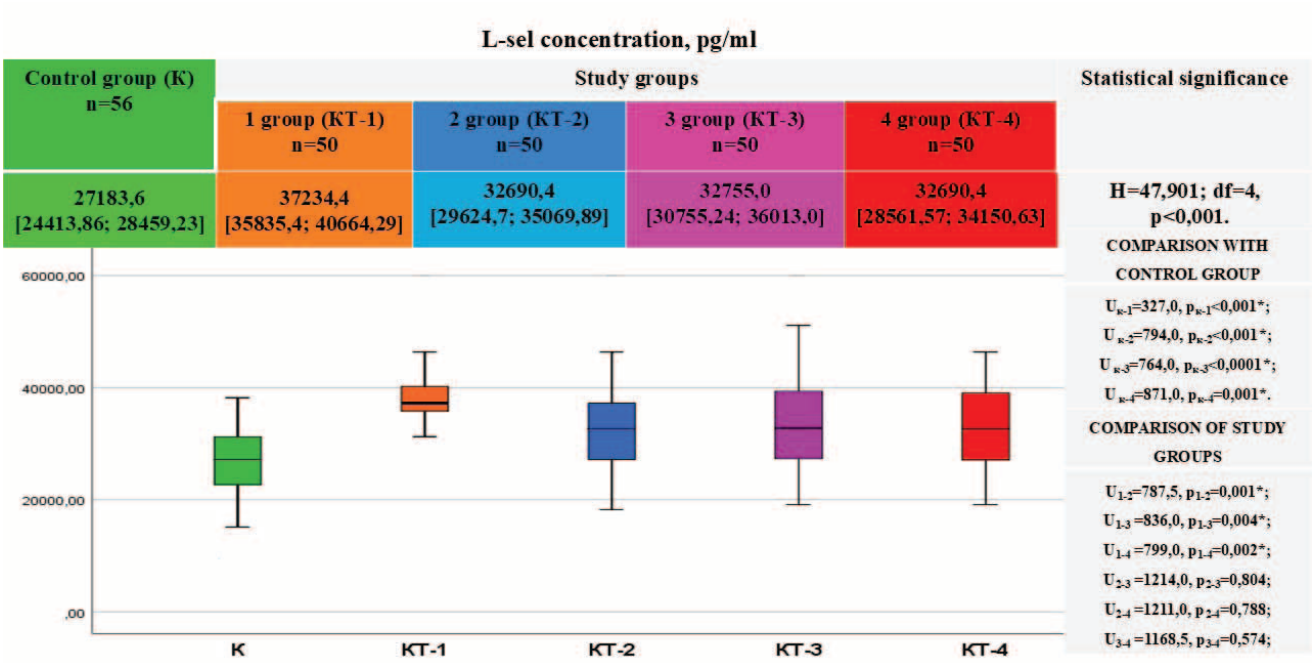

Figure 9. *The concentration of L-selectin intercellular adhesion molecules in the blood of patients in the studied groups*
Note: see figure 1

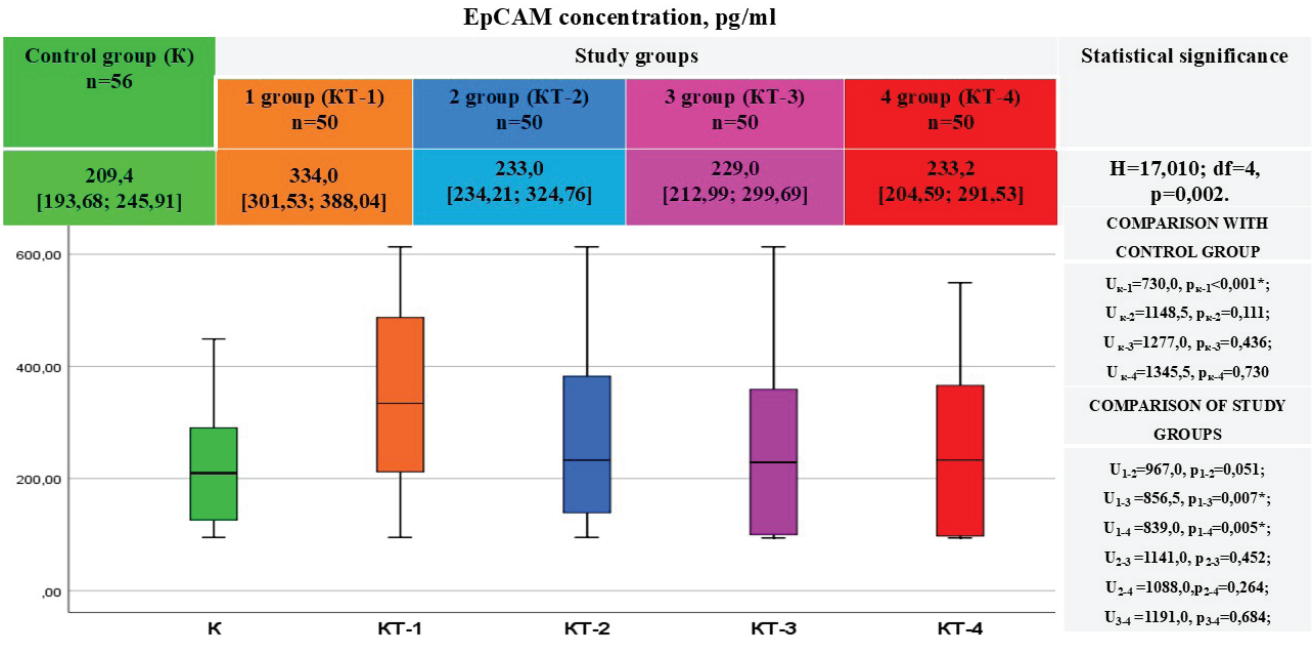

Figure 10. *The concentration of EPCAM intercellular adhesion molecules in the blood of patients in the study groups*
Note: see figure 1

the method of elimination were used to identify the factors with the highest confidence, including intercellular adhesion molecules. This model was converted into a calculator, which can be used in practical healthcare; and details will be provided later in publications.

Conclusions. Thus, patients with past coronavirus infection with lung involvement have higher concentrations of intercellular adhesion molecules from all superfamilies. Higher levels of intercellular adhesion molecules in study subjects prove the presence of endotheliosis and correlate with the degree of pulmonary tissue involvement, including recovery.

Вклад авторов:

Все авторы внесли существенный вклад в подготовку работы, прочли и одобрили финальную версию статьи перед публикацией

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Authors' contributions:

All authors made significant contributions to the preparation of the work, read and approved the final version of the article before publication

Karachenova A.M.: author's contribution to the development of the concept and design of the study, collection, analysis and interpretation of data, analysis of literature on the research topic, scientific editing, significant contribution to research work

Romanova E.N.: author's contribution to the development of the concept and design of the study, analysis of literature on the research topic, scientific editing, approval of the final text of the article, significant contribution to research work

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КЛИНИЧЕСКАЯ ХАРАКТЕРИСТИКА НЕЙРОФИБРОМАТОЗА 1-ГО ТИПА В РЕСПУБЛИКЕ БАШКОРТОСТАН

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Clinical Characteristics of Neurofibromatosis Type 1 in the Republic of Bashkortostan

Резюме

Актуальность. Нейрофиброматоз 1-го типа (НФ1) — это наследственный опухолевый синдром, встречающийся с частотой 1:3164 населения в мире. Болезнь характеризуется тяжелыми клиническими проявлениями в виде множественных кожных и подкожных опухолей, плексиформных нейрофибром, скелетных аномалий, когнитивных расстройств и различных осложнений. **Цель исследования.** Определение частоты встречаемости НФ1 в Республике Башкортостан и ее динамики, клинических особенностей НФ1 для совершенствования организационных и лечебно-диагностических подходов при оказании медицинской помощи пациентам с НФ1. **Материал и методы.** Проведено клинико-эпидемиологическое исследование больных НФ1 в Республике Башкортостан и сравнительный анализ с данными за 2009 и 2021 годы. **Результаты.** В Республике Башкортостан зарегистрировано 544 больных НФ1 из 433 семей в возрасте от 1 до 85 лет (средний возраст 30 лет и 7 месяцев), частота встречаемости составила 1:7407 человек. Характерные для НФ1 пигментные пятна определены у всех пациентов, кожные и подкожные нейрофибромы у 58 %, плексиформные нейрофибромы — у 7 %, сколиоз — у 17,4 %. Трудности в обучении выявлены у 14 %, эпилепсия — у 3,7 %, гидроцефалия — у 4 %, глиомы зрительных нервов — у 6 %, опухоли головного мозга — у 4 % больных. **Обсуждение.** Сравнительный анализ особенностей клинических проявлений НФ1 у больных из Республики Башкортостан с мировыми данными показал достоверно более редкое выявление нейрофибром, узелков Лиша, глиом зрительных нервов, нарушений интеллекта и психологических расстройств. Количество пациентов с НФ1 в республике увеличилось в 2,3 раза за 15 лет и на 35 % за последние 3 года. Более того, 4 больных с плексиформными нейрофибромами получают ингибитор митоген-активируемой протеинкиназы, показавший свою эффективность. **Заключение**. Полученные результаты свидетельствуют о повышении количества зарегистрированных случаев НФ1 за последние годы и необходимости мультидисциплинарного подхода в исследовании пациентов в связи с достоверно низкой частотой регистрации характерных симптомов болезни.

Ключевые слова: *ген NF1, диагностика, лечение, нейрофиброматоз 1-го типа, нейрофибромы, опухоли, частота встречаемости.*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

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Abstract

Relevance. Neurofibromatosis type 1 (NF1) is a hereditary tumor syndrome occurring with a frequency of 1:3164 of the world's population. The disease is characterized by severe clinical manifestations such as multiple cutaneous and subcutaneous tumors, plexiform neurofibromas, skeletal abnormalities, cognitive disorders and various complications. **The aim of the study.** To determine the frequency of NF1 in the Republic of Bashkortostan and its dynamics, clinical features of NF1 to improve organizational and therapeutic and diagnostic approaches in providing medical care to patients with NF1.

Material and methods. A clinical and epidemiological study of NF1 patients in the Republic of Bashkortostan and a comparative analysis with data for 2009 and 2021 were conducted. **Results.** In the Republic of Bashkortostan, 544 patients with NF1 from 433 families aged 1 to 85 years (average age 30 years and 7 months) were registered, the incidence rate is 1:7407 people. Pigment spots were identified in all patients, cutaneous and subcutaneous neurofibromas in 58 %, plexiform neurofibromas in 7 %, scoliosis in 17.4 %. Learning difficulties were identified in 14 %, epilepsy in 3.7 %, hydrocephalus in 4 %, optic nerve gliomas in 6 %, and brain tumors in 4 % of NF1 patients from the republic. **Discussion.** A comparative analysis of the characteristics of NF1 in patients from the Republic of Bashkortostan with global data showed a significantly rarer detection of neurofibromas, Lisch nodules, optic nerve gliomas, intellectual disabilities and psychological disorders. The number of patients with NF1 in the republic has increased by 2.3 times in 15 years and by 35 % in the last 3 years. Moreover, 4 patients with plexiform neurofibromas are receiving a mitogen-activated protein kinase inhibitor, which has proven its effectiveness. **Conclusion**. The obtained results indicate an increase in the number of registered cases of NF1 in recent years, but the need for a multidisciplinary approach in the study of patients due to the reliably low frequency of registration of characteristic symptoms of the disease.

Keywords: *NF1 gene, diagnosis, treatment, neurofibromatosis type 1, neurofibromas, tumors, incidence.*

Conflict of interests
The authors declare no conflict of interests

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Conformity with the principles of ethics
All patients signed an informed consent. The study protocol was approved at a meeting of the Local Ethics Committee (protocol No. 5 dated December 7, 2009)

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GTP — guanosine triphosphate, NF1 — neurofibromatosis, type 1, RB — Republic of Bashkortostan, CALM — café-au-lait macules, GCP — Good Clinical Practice, GRD — GAP-related domain, MPNST — malignant peripheral nerves sheath tumour, NIH — National Institutes of Health

Introduction

Neurofibromatosis, type 1 (NF1) is one of the most common hereditary tumour syndromes with autosomal dominant mode of inheritance, the global incidence of which is 1 : 3,164 people (the value varies between 1 : 2,132 and 1 : 4,712 depending on the country) [1]. This condition is driven by heterozygous mutations in *NF1* tumour suppressor gene, which encodes neurofibromin, GTP-activating protein, comprising 2,818 amino acids and containing a domain, downregulating the activity of Ras protooncogenes, called GRD (GAP-related domain) [2]. Approximately a half of all NF1 cases are sporadic due to new mutations in gametal cells in parents (80 % are found in spermatozoa) [3].

NF1 manifests with specific café-au-lait pigmented spots (CALM, café-au-lait macules) measuring over 5 mm in diameter in pre-puberty and over 15 mm in post-puberty (99 %), or inguinal or axillary freckles, iris hamartomas (Lisch nodules), cutaneous and subcutaneous neurofibromas, optic nerve gliomas and plexiform neurofibromas, as well as typical bone abnormalities (sphenolateral bone dysplasia, cortex thinning and/or long bone pseudoarthrosis). Where two or more of these signs are present, clinical NF1 is diagnosed as per the guidelines of the National Institutes of Health (NIH). Where NF1 is confirmed in close relatives, one sign of the disease is enough [4]. NF1 is associated with complete penetration, therefore molecular genetic verification is not mandatory, and the condition is diagnosed on the basis of the above criteria in 90 % of cases in patients under 7 years old and in 100 % of cases in patients under 19 years old [3].

According to global references, CALM is observed in 96.5 % of patients with NF1, while inguinal or axillary freckles are recorded in 90 % of cases. Cutaneous and/or subcutaneous neurofibromas are diagnosed in over 99 %, iris hamartomas — in 70 %, plexiform neurofibromas — in a half of NF1 patients [4]. The incidence of optic nerve gliomas in this disease is 27 %, brain tumours — 10 %, dropsy of brain — 7.7 % [5]. Malignant peripheral nerves sheath tumours (MPNST) are very rare and aggressive neoplasms in general population, while they are observed in 13 % of NF1 cases. Usually, these tumours are a result of canceration of existing plexiform neurofibromas [6].

Cognitive disorders in NF1 patients are diffuse and can be observed throughout their lives [3]. According to meta-analysis results, convulsive disorder is recorded in 8.1 % of NF1 patients (of which: generalised tonic-clonic epilepsy — 16.8 %, focal fits — 54.2 %; with one or two anticonvulsants, absence of seizures was observed in 68.5 %; median age: 3.5 to 12 years old) [7]. Impaired mental capacity, resulting in learning difficulties, is diagnosed in 40 % of NF1 cases; the mean IQ value is 85–90. Autism spectrum disorders are observed in 25–30 % of NF1 patients, while attention deficit/hyperactivity disorder is recorded in 40 % [3].

NF1 patients have locomotor disorders. According to a meta-analysis, approximately 26.6 % of NF1 patients have scoliosis. It usually develops in the early childhood and affects the thoracic spine. No reliable correlation between scoliosis and NF1 genotype has been identified. In terms of efficacy and safety, spinal fusion and growing rods demonstrated the best results in scoliosis management in NF1 patients [8]. On the average,

5 % of NF1 patients globally have pseudoarthrosis [4], 24 % of them have short stature [9]. Of interest is the description of the clinical presentation of NF1 in patients in the Republic of Bashkortostan and comparison of the results with the results of academic publications and meta-analyses from various countries, as well as previous data for this region. The results of the analysis can help in identifying the required areas of medical care improvement.

Study Objective

To identify the incidence of NF1 in the Republic of Bashkortostan and its changes, clinical characteristics of NF1 and comparison of results with available global data in order to improve organisational, treatment and diagnostic approaches to the management of NF1 patients.

Materials and Methods

The analysis included data on NF1 patients from the Republic of Bashkortostan (RB) registered with a genetics specialist at the Republican Medical and Genetics Centre with confirmed NF1. All the studies were performed in accordance with the requirements of biomedical ethics and GCP (Good Clinical Practice). We have studied clinical manifestations of NF1 in patients in RB and the comparison of the data with global results, as well as published results of studies conducted in RB in 2009 and 2021. Statistical processing of qualitative binary data was performed using an interactive 2×2 cross table with calculation of relation statistics (Pearson's χ^2) with Yates' correction for continuity developed by V. P. Leonov, as well as analysis of four-fold cross tables; see https://med-statistic.ru/calculators/calchi.html.

Results

In the Republic of Bashkortostan, there are 544 NF1 patients aged 1 to 85 years old (mean age: 30 years 7 months) in 433 families, i. e. 1 : 7,407 people, with uneven area distribution (Figure 1). Despite the fact that these figures differ from the global values more than two times (1 : 3,164 population [1]), if comparing to the 2009 national data (238 NF1 patients in 192 families, incidence: 1 : 17,000 [10]), the number of registered NF1 patient rose 2.3-fold, evidencing higher utilisation of genetics specialist services by patients. Over the past three years vs. the 2021 data (401 patients in 321 families, incidence: 1 : 10,103 [11]), the number of registered NF1 cases increased by 35 % (Figure 2).

In the RB, there are 299 sporadic cases (55 %) and 45 % of hereditary NF1 cases; male-to-female ratio is approximately 1 : 1 (52 % of females and 48 % of males), corresponding to the global statistics [3] and results of previous NF1 studies conducted in the RB [10, 11]. Pigmented spots were observed in all NF1 patients; 314 patients (58 %) had cutaneous or subcutaneous neurofibromas, which is lower than the global value (99 %) [4]. This difference can be explained by the fact that 42 % of patients, who did not present with cutaneous or subcutaneous neurofibromas, had their NF1 diagnosed from the family history (NF1 in either parent). Also, some patients had CALM + plexiform neurofibromas or CALM + optic nerve gliomas without visible cutaneous or subcutaneous neurofibromas, which meets the criteria set forth by the NIH for NF1 diagnosis. Of them, 112 people had focal neurofibromas, whereas the majority of patients (64 %) had multiple neurofibromas. Although the global incidence of MPNST in NF1 patients is 13 % [6], there were no cases of this neoplasm among 544 NF1 patients in the RB. Learning difficulties were diagnosed in 78 patients in the RB (14 %), which also differs from study results in other countries (40 %) [3].

Some NF1 patients in the RB had brain damage; 20 (3.7 %) NF1 patients had epilepsy, 23 (4.23 %) — dropsy of brain, 22 (4 %) — cerebral cysts, 21 (3.86 %) — brain growth, and 34 (6.25 %) — optic nerve gliomas. Lisch nodules were described just in 5 patients (1 %). Scoliosis was diagnosed in 95 NF1 patients (17.4 %), short stature — in 75 (13.8 %), lower-leg bones pseudoarthrosis — in 15 (3 %).

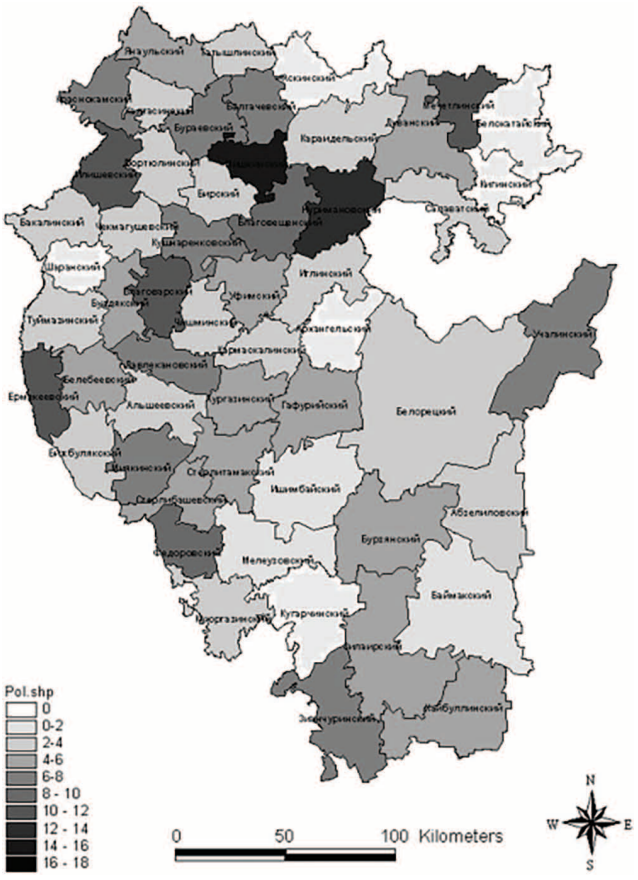

Figure 1. *Distribution map of patients with NF1 in the Republic of Bashkortostan*

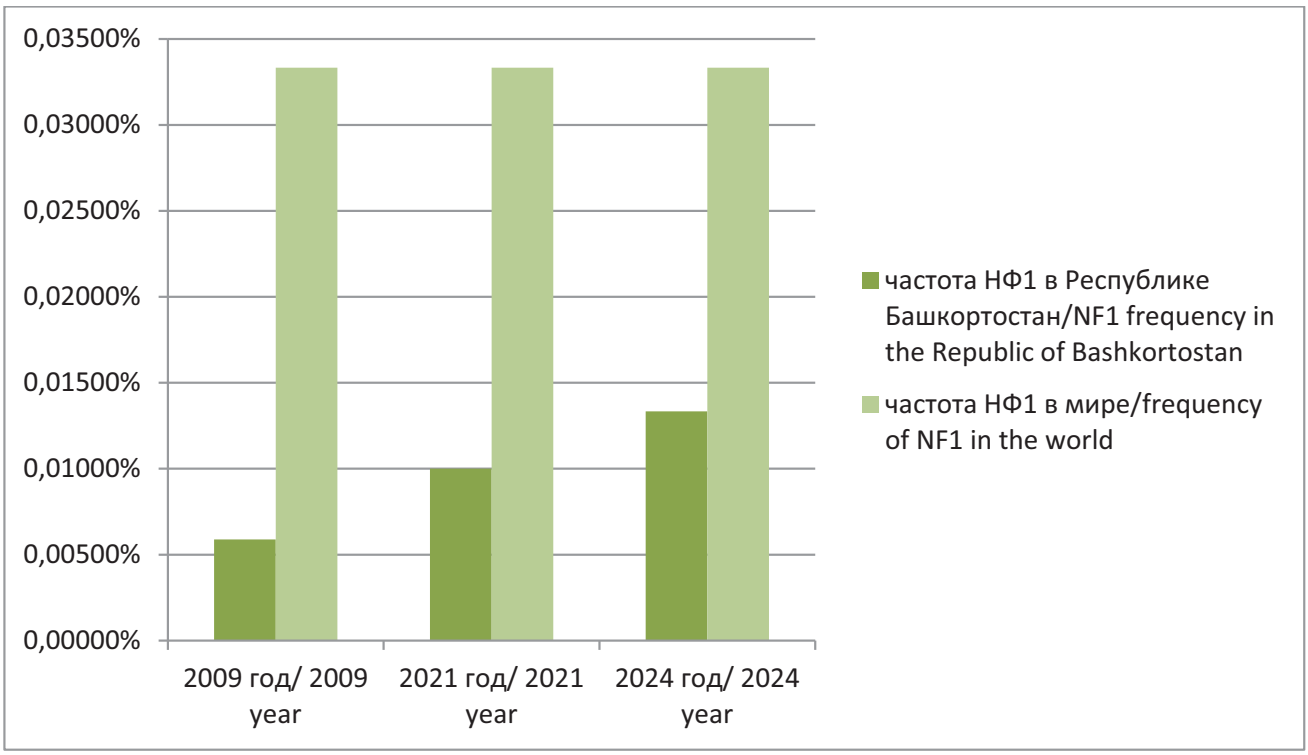

Figure 2. *Scheme of the dynamics of the increase in the frequency of occurrence of NF1 in the Republic of Bashkortostan and in the world*

Plexiform neurofibromas were described in 38 (7 %) NF1 patients in the RB. Four patients are currently undergoing efficient target therapy with mitogenic-activated protein kinase inhibitor (selumetinib [12]) within the Circle of Goodness program. These are a 10-year-old boy (inherited NF1 from his mother, selumetinib 30 mg/day) with facial plexiform neurofibroma; a 6-year-old boy (inherited NF1 from his father, selumetinib 30 mg/day) with oropharynx plexiform neurofibroma; a 12-year-old girl (inherited NF1 from her mother, selumetinib 50 mg/day) with hypopharynx plexiform neurofibroma; and a 6-year-old boy (sporadic case, selumetinib 20 mg/day) with plexiform neurofibroma of his supraclavicular region and mediastinum. With the therapy, the four NF1 patients had improvements over a one-year follow-up period: reduction in the size of plexiform neurofibromas.

Discussion

A comparative analysis of clinical manifestations of NF1 in patients in the Republic of Bashkortostan (Table 1) shows statistically significantly lower incidence of cutaneous and subcutaneous neurofibromas, Lisch nodules, plexiform neurofibromas, optic nerve gliomas, short stature and impaired mental capacity vs. global data [3–6, 9]. These data are likely to be a result of lack of specialised consultations to describe and diagnose the pathology. An analysis of medical records demonstrated that only eight NF1 patients in the RB were consulted by an eye specialist; following consultations, Lisch nodules were diagnosed in 63 %, which is also less than the global data [4], however, the difference is not statistically significant ($\chi^2 = 808$; $p = 0.369$). According to the data from medical records, oncologists consulted 168 (31 %) NF1 patients, mainly those with severe signs of disease, e.g., recorded plexiform neurofibromas, optic nerve gliomas, brain tumours, because these patients require a special approach and antitumour management. There are no data on consultations by surgeons, psychiatrists and psychologists in patient medical records, and such consultations need to be included into examinations of NF1 patients. More reliable pathology identification requires a thorough examination, consultation by an eye specialist, and NF1 signs, patient height and weight should be recorded in medical records; questionnaires should be used to identify any mental impairment.

Also, absence of MPNST, autism spectrum disorders and attention deficit disorder in NF1 patients in the RB is statistically different. It shows the need for whole-body MRI and consultation by a cancer specialist to diagnose MPNST, and consultation by a psychiatrist and psychologist to diagnose mental disorders. The incidence of CALM, brain tumours, dropsy of brain, epilepsy and skeleton abnormalities correlates with the data presented by other authors [4, 5, 7, 8].

Table 1. *Comparative characteristics of neurofibromatosis type 1 features in patients from the Republic of Bashkortostan with world data*

Clinical features	Frequency of occurrence in NF1-patients from RB in %	Frequency of occurrence in patients worldwide in % [author]	χ2 test; p-value at 1 degree of freedom
cutaneous and subcutaneous neurofibromas	58 %	99 % [4]	$\chi^2 = 49,8$; $p<0,001$
Lisch nodules	1 %	70 % [4]	$\chi^2 = 103,9$; $p<0,001$
plexiform neurofibromas	7 %	50 % [4]	$\chi^2 = 45,37$; $p<0,001$
MPNST	0 %	13 % [6]	$\chi^2 = 13,9$; $p<0,001$
optic nerve gliomas	6,25 %	27 % [5]	$\chi^2 = 16,004$; $p<0,001$
brain tumor	3,86 %	10 % [5]	$\chi^2 = 2,765$; $p = 0,097$
hydrocephalus	4,23 %	7,7 % [5]	$\chi^2 = 1,418$; $p = 0,234$
epilepsy	3,7 %	8,1 % [7]	$\chi^2 = 1,418$; $p = 0,234$
scoliosis	17,4 %	26,6 % [8]	$\chi^2 = 2,914$; $p = 0,088$
short stature	13,8 %	24 % [9]	$\chi^2 = 3,25$; $p = 0,072$
pseudoarthrosis	3 %	5 % [4]	$\chi^2 = 0,521$; $p = 0,471$
learning difficulties	14 %	40 % [3]	$\chi^2 = 27,022$; $p<0,001$
autism spectrum disorders	0 %	28 % [3]	$\chi^2 = 32,558$; $p<0,001$
attention deficit hyperactivity disorder	0 %	40 % [3]	$\chi^2 = 50$; $p<0,001$

Table 2. *Comparative analysis of clinical manifestations of NF1 in male and female patients from the Republic of Bashkortostan*

Clinical features	Frequency of occurrence in male NF1-patients with from RB, n=259	Frequency of occurrence in female NF1-patients with from RB, n=285	χ2 test; p-value at 1 degree of freedom
neurofibromas	149 (58 %)	165 (58 %)	$\chi^2 = 0,007$; $p = 0,932$
Lisch nodules	3 (1,16 %)	2 (0,7 %)	$\chi^2 = 0,311$; $p = 0,578$
plexiform neurofibromas	18 (7 %)	20 (7 %)	$\chi^2 = 0,001$; $p = 0,976$
optic nerve gliomas	19 (7,34 %)	15 (5,3 %)	$\chi^2 = 0,995$; $p = 0,319$
brain tumor	12 (4,6 %)	9 (3,1 %)	$\chi^2 = 0,796$; $p = 0,373$
hydrocephalus	14 (5,4 %)	9 (3,1 %)	$\chi^2 = 1,693$; $p = 0,194$
epilepsy	11 (4,3 %)	9 (3,1 %)	$\chi^2 = 0,455$; $p = 0,501$
scoliosis	47 (18,15 %)	48 (16,8 %)	$\chi^2 = 0,160$; $p = 0,689$
short stature	27 (10,4 %)	48 (16,8 %)	$\chi^2 = 3,480$; $p = 0,063$
pseudoarthrosis	4 (1,54 %)	11 (3,9 %)	$\chi^2 = 2,713$; $p = 0,100$
learning difficulties	46 (17,7 %)	32 (11,23 %)	$\chi^2 = 4,714$; $p = 0,030$

A comparative analysis of recorded clinical manifestations of NF1 in patients in the RB this year vs. 2009 [10] and 2021 [11] did not demonstrate significant changes in the incidence of clinical manifestations of the disease. In order to determine possible impact of sex differences on the incidence of NF1 symptoms, a comparative analysis of clinical signs in male and female patients was performed (Table 2). Significant differences were found only in terms of learning difficulties (learning difficulties are more frequent in male patients).

According to the analysed data from the study of NF1 patients in the RB, there is no information on the presence of autism spectrum disorders, attention deficit/hyperactivity disorder; however, the global incidence of these conditions among patients is 30–60 % and 25–30 %, respectively [3]. It demonstrates the need to refer NF1 patients to psychologists, neurologists and psychiatrists for timely diagnosis and management of neurological and mental disorders. It is even more important, since multiple neurofibromas on patients' bodies (64 % of examined NF1 patients) have mental implications [3] (Figure 3). Medical records of NF1 patients in the RB do not contain any data on mental disorders, such as depression, anxiety or distress; however, scientific references suggest that these conditions are not uncommon among NF1 patients. For instance, clinical depression is diagnosed in 19 % and anxiety disorders are observed in 15 % of NF1 patients [13]. Their diagnosis and management by a psychologist will help improve the quality of patient's life significantly. Given a new therapy of plexiform neurofibromas — mitogenic-activated protein kinase inhibitor (selumetinib), this product should be used more widely, since plexiform neurofibromas were diagnosed in 38 NF1 patients in the RB. Indications for selumetinib therapy currently include documented plexiform neurofibroma, and the condition of use is genetically confirmed NF1 (heterozygous mutation in gene *NF1*) [14]. Efficiency criteria of the medicinal product are tumour reduction, which, according to a meta-analysis [12], was observed in 75.3 %. The majority of adverse reactions to selumetinib were mild; the most common reactions were diarrhoea and vomiting [12].

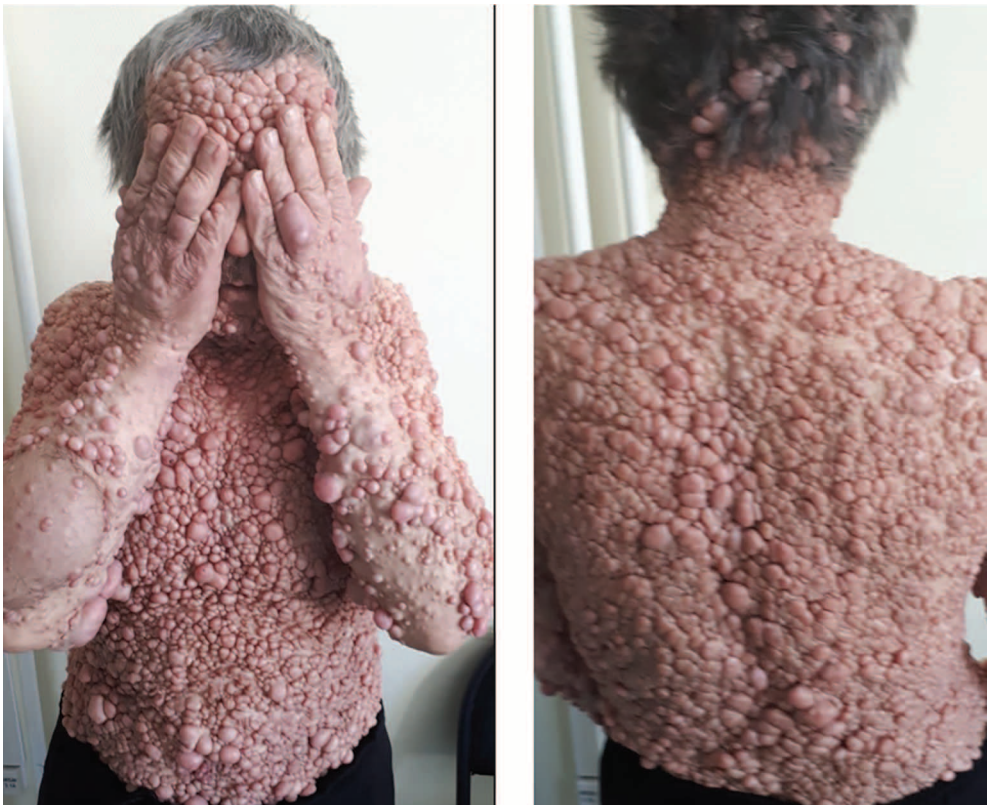

Figure 3. *A patient from the Republic of Bashkortostan with multiple cutaneous and subcutaneous neurofibromas (the author's photo was taken with the consent of the patient and his relatives).*

Conclusion

We have conducted a clinical and epidemiological study of NF1 patients in the Republic of Bashkortostan. As a result, we have identified the incidence of the disease, which was 1 : 7,407, i.e. significantly lower than the global figures (1 : 3,164); however, the incidence is significantly higher than in previous periods. The ration between male and female NF1 patients was 1 : 1, sporadic cases accounted for 55 %. The clinical characteristics of NF1 patients in the Republic of Bashkortostan show comparable incidence of neurofibromas, brain tumours, dropsy of brain, epilepsy, scoliosis, and pseudoarthrosis if compared to the global data. Significantly lower incidence of plexiform neurofibromas, optic nerve gliomas, short stature and impaired mental capacity have been observed in NF1 patients in the Republic of Bashkortostan. It has been established that the incidence of clinical manifestations of the disease in male and female patients is comparable, and men have learning difficulties more often. An analysis of patient examination in the Republic of Bashkortostan shows the need for patient referral to all-body MRI for timely diagnosis of plexiform neurofibromas, because these tumours are an indication for mitogenic-activated protein kinase inhibitor therapy. Since the diagnosis of NF1 is based on NIH-approved clinical criteria, a multidisciplinary approach in disease diagnosis would be advisable. Also, consultations by specialists can help in more precise diagnosis of NF1, since this condition shares clinical presentation with other diseases [15]. For instance, a consultation by an eye specialist can help diagnose Lisch nodules, the presence of which is described just in 1 % of all NF1 patients in the RB. A consultation by a neurosurgeon allows identifying an approach to the management of dropsy of brain, tumours and cerebral cysts. Referral of paediatric NF1 patients to an orthopaedist can ensure early diagnosis and correction of skeleton abnormalities. Resection of multiple cutaneous and subcutaneous neurofibromas is possible in a surgical ward, especially in those using a surgical laser [14]. In order to diagnose and treat autism spectrum disorders, attention deficit/hyperactivity disorder, cognitive deficit and distress caused by numerous psycho-traumatic tumours, it is advisable to refer patients to psychologists and psychiatrists. These measures will significantly improve the quality of diagnosis and management of NF1 patients and make their life better.

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КОРРЕЛЯЦИЯ МЕЖДУ ПОРАЖЕНИЕМ МЕЛКИХ И КРУПНЫХ СОСУДОВ У ПАЦИЕНТОВ С САХАРНЫМ ДИАБЕТОМ 2-ГО ТИПА

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Correlation Between Microvascular and Macrovascular Affection in Type 2 Diabetes Mellitus

Резюме

Цель работы: изучить возможную взаимосвязь между макрососудистыми заболеваниями, особенно атеросклерозом, и нарушениями микроциркуляции у пациентов с СД2, а также оценить взаимосвязь между уровнем глюкозы крови и поражением мелких и крупных сосудов. **Пациенты и методы**: В исследовании приняло участие 150 пациентов: 100 пациентов с СД2 и контрольная группа из 50 участников. Все участники прошли сбор анамнеза и клиническое обследование, а также сдали кровь на биохимический анализ, включая определение уровней гликированного гемоглобина (HBA1c), глюкозы плазмы натощак (FPG), постпрандиального уровня глюкозы через 2 часа (2h-PG), триглицеридов (TG), общего холестерина (TC), ЛПВП и ЛПНП. Видео-капилляроскопию ногтевого ложа (NVC) проводили с целью оценить морфологию капилляров ногтевого ложа, диаметр артерий и вен, изменения длины капилляров и размер петли, наличие или отсутствие капиллярного кровотечения, кровоподтеков, рубцевания, дефектных и крупных капилляров. Для оценки таких изменений использовали полуколичественную шкалу (0–3). Все участники прошли дуплексное исследование сонной артерии для измерения толщины слоя интима-медиа в общей сонной артерии (ТИМ). **Результаты.** Пациенты с СД2 имели существенно большее значение ТИМ по сравнению с контрольной группой. Отмечались более частые случаи отклонений в морфологии капилляров, кровотечений, рубцевания и дефектов капилляров. Модифицированный балл NVC у пациентов с СД2 составил >1 по сравнению с контрольной группой. Кроме того, у пациентов с СД2 наблюдались более высокие показатели частоты кровоподтеков, разветвлений, пересечений и штопоровидных капилляров, большие петли и укорочение капилляров. Зарегистрировано значительное увеличение значений ТИМ слева и справа в группе участников с СД, а модифицированный балл NVC составил >1. **Заключение.** Отмечена тесная связь между атеросклерозом и нарушением микроциркуляции. Видео-капилляроскопию можно использовать для оценки нарушений микроциркуляции до обнаружения атеросклероза по результатам дуплексного исследования сонной артерии.

Ключевые слова: *диабет, видео-капилляроскопия ногтевого ложа, дуплексное исследование сонной артерии, атеросклероз, микроциркуляция*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

Источники финансирования

Авторы заявляют об отсутствии финансирования при проведении исследования

Соответствие принципам этики

Исследование одобрено этическим комитетом учреждения (медицинский факультет, Каирский университет (Египет) — Протокол № 132701-2019). Все участники предоставили согласие на участие в исследовании.

Ограничения исследования

Рекомендуется проводить исследование в большей группе для лучшего понимания и корреляции с ретинопатией как микрососудистым осложнением. Кроме того, требуется последующее наблюдение для лучшей оценки взаимосвязи между микроциркуляторными изменениями и макрососудистыми осложнениями.

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Abstract

Aim of the work: to explore the possible relation between macrovascular disease especially atherosclerosis and microcirculation abnormalities in patients with T2DM and, to assess any relationship between blood glucose level, microvascular and macrovascular affection. Patients and methods: the study recruited 150 participants; 100 patients with T2DM and 50 controls. All participants underwent history taking, clinical examination, biochemistry testing including HBA1c, FPG, 2h-PG, TG, TC, HDL, and LDL. Nailfold video capillaroscopy (NVC) was performed to evaluate morphology of the nailfold capillaries, arterial and venous limb diameter, alteration in Capillary length and loop diameter, presence or absence of capillary hemorrhage, extravasation, scarring, scanty and large capillaries. To score these alterations, a semi-quantitative rating scale (0–3) was used. Carotid duplex was done to all participants to measure the intima media thickness in the common carotid artery (CIMT). Results: Subjects with T2DM showed significantly increased CIMT when compared with controls. There were a significantly higher frequencies of abnormal capillary morphology, hemorrhage, scarring and scanty capillaries, Modified NVC score>1 in T2DM. In comparison to the control group, they also exhibited noticeably greater rates of extravasation, branching, crossed, and corkscrew-shaped capillaries, larger loops, and decreased capillary length. There was significantly higher left and right CIMT in the group of diabetics with Modified NVC score >1. Conclusion: A significant relationship was found between atherosclerosis and microcirculation abnormalities. Videocapilloroscopy could be used to assess microcirculatory abnormalities before detection of atherosclerosis by carotid duplex.

Key words: *diabetes, nailfold videocapillaroscopy, carotid duplex, atherosclerosis, microcirculation*

Conflict of interests

The authors declare no conflict of interests

Sources of funding

The authors declare no funding for this study

Conformity with the principles of ethics

The study was approved by the institutional ethics committee (Faculty of Medicine, Cairo University (Egypt) Protocol No. 132701-2019). All participants provided consent to participate in the study.

Limitations

A larger study group is advised for better understanding and correlation with retinopathy as a microvascular complication, also follow up is needed for better assessment of the relation of microcirculatory changes and macrovascular complications.

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ADA: American Diabetes Association, ASE: American Society of Echocardiography, BMI: Body mass index, CIMT: Carotid intima media thickness, DPP4i: dipeptidyl peptidase 4 inhibitors, EDV: End Diastolic Velocity, ESC: European Society of Cardiology, FBG: Fasting blood glucose, HBA1c: Hemoglobin A1c, HDL: High density lipoprotein, 2h-PG: 2-hour postprandial blood glucose, HTN: Hypertension, ICA: Internal carotid artery, LDL: Low density lipoprotein, Lt: Left, NVC: Nailfold videocapilloroscopy, OGTT: Oral glucose tolerance test, PSV: Peak Systolic Velocity, RBS: Random blood sugar, RI: Resistive index, Rt: Right, T2DM: Type 2 Diabetes mellitus, SD: Standard deviation, TC: Total cholesterol, TG: Triglycerides, WC: Waist circumference

Introduction

T2DM is a metabolic disorder that affects quality of life and represents a burden on society because it is accompanied with many complications mainly vascular complications [1].

About one-third to one-half of diabetic patients experience vascular complications, which can be classified as macrovascular or microvascular). These complications cause damage and failure of various organs [2].

Hyperglycemia is the main cause of microvascular and macrovascular affection. Both types of vascular complications seem to be interconnected but the relation between microvascular, macrovascular complications and chronic hyperglycemia is not clear yet [3].

The artery disease known as atherosclerosis is typified by the buildup of fatty plaques on the inner walls of the vessels. It takes many years for it to develop. Atherosclerotic vascular alterations are linked to both macrovascular and microvascular diabetes problems. B-mode ultrasound is used to measure CIMT, a non-invasive marker of subclinical atherosclerosis. Numerous research has demonstrated a link between CIMT and atherosclerosis [4].

The aim of this work was to explore the possible relation between macrovascular disease variables and microcirculation abnormalities in patients with T2DM. Also, to assess any relationship between blood glucose level, microvascular and macrovascular affection.

Patients and methods

The study recruited 150 participants from the outpatient clinic of DM at Kasr Alainy teaching Hospital divided into two groups age and sex matched: control group which included 50 healthy individuals, and T2DM group which included 100 patients suffering from T2DM based on the ADA criteria diagnosed for at least one year.

The T2DM group was further divided according to glycemic control into two groups, one with HBA1c<7 and the other one with HBA1c>7; to study the relation between glycemic control and microcirculatory changes.

T2DM group was also subdivided into dyslipidemic and non dyslipidemic group to assess the role of dyslipidemia in microcirculatory changes.

Sample size was calculated using STATA 16.

All participants underwent complete medical history taking, thorough clinical examination , biochemical testing was done including HbA1c (%), FPG (mg/dL), 2h-PG (mg/dl) TG (mg/dL), Total Cholesterol (TC) (mg/dL), HDL (mg/dL), and LDL (mg/dL),Imaging in the form of nailfold Capillaroscopy to detect microvascular affection, and carotid doppler ultra-sonography to detect macrovascular affection. They study follows the principles outlined in the Helsinki Declaration of 1964 and its later amendments. The study was approved by institutional ethical committee (faculty of Medicine, Cairo University, Egypt, 2019). A consent was taken from the participants.

Inclusion criteria included adult patients above 30 years old with type 2 diabetes diagnosed for at least one year based on American Diabetes Association criteria [5] as follows: a FPG level of ≥ 126 mg/dL, a 2-h Plasma Glucose (2h-PG) level of ≥ 200 mg/dL in the 75-g OGTT, or a RBG level of ≥ 200 mg/dL and/or HbA1c level ≥ 6.5 %.

Exclusion criteria were any evidence of cancer, active liver disease, current pregnancy, active infection, very poorly controlled heart disease, pulmonary disorders, current or previous tobacco smoking or severe impairment of the renal function, individuals with injuries within the nail fold as a result of aesthetic procedures or nail polish, as well as those exhibiting symptoms of any vascular collagen disease.

None of the patients previously received antihypertensive or lipid lowering drugs.

A nailfold Capillaroscopy examination was performed on each participant utilising a video Capillaroscopy. The optical microscope was connected to a digital camera and computer.

The participant sat calm on the chair in front of the machine. The procedure was explained for him. To increase the translucency, a drop of immersion oil was applied to the finger's nail fold.

The following capillaroscopic properties were evaluated for every image: morphology of the nailfold capillaries (considered normal when there is uniform distribution of capillaries resembling hairpins (comb-like structure) morphological anomalies include branched capillaries, cork screw, and crossed), large capillary (defined by Marique's as 4-10 increase in capillary size and The diameter of the arterial and venous limbs ranges from 7 to 17 μm and 11 to 20.6 μm, respectively, while the capillary width of expanded capillaries is at least 90 to 150 μm (0.090 to 0.150 mm), alteration in Capillary loop diameter (normal values 8 to 14 μm, enlarged loop > 20 μm,), alteration in Capillary length (normal length 200 to 500 μ and increased length, or reduced length) [6-10] and presence or absence of capillary hemorrhage, extravasation and scarring, scanty capillaries.

According to earlier research, a semi-quantitative grading scale was used to grade these modifications [6] score 0, no significant changes, score 1, few (< 4 alterations), score 2, some (between 4 and 6 alterations) and score 3, frequent (> 6 alterations/linear mm).

We categorized our subjects in 2 groups: modified NVC score >1: includes Patients with abnormal capillaroscopic pattern with existence of ≥ 4 abnormal parameters (score 2,3) and modified NVC score ≤ 1comprises individuals who have a uniform distribution of comb-like, hairpin-shaped capillaries and no discernible alterations (score 0).

Participants with suspicious capillaroscopic pattern (score 1) with existence of ≤ 3 abnormal parameters (non-specific morphological abnormalities) [6, 12-14].

Numerous research studies have demonstrated that a microcirculation score more than one indicates a clinically severe impairment [6, 12, 13, 15].

Modified nailfold video capillaroscopy (NVC) attributes were quantified using the NVC score in accordance with a number of criteria.

The report by Barchetta et al. is where the scoring standards were adjusted [6]. All collected data were then documented and subjected to statistical analysis.

An HD5000 system (Philips Ultrasound, Bothell, Washington) equipped with a 7.5- megahertz (MHz) linear array probe to assess the maximum thickness of IMT and a 5-MHz linear array probe to assess the RI of ICA was used to perform the duplex sonographic evaluation.

The patient was lying flat in bed. The IMT was measured in the common carotid artery 2 cm just before common carotid artery bifurcation and also common and internal carotid arteries were screened for any atherosclerotic plaques. Peak Systolic Velocity (PSV) and End Diastolic Velocity (EDV) were be calculated of both internal carotid arteries [16].

Spectral analysis: This makes it possible to estimate the blood flow rate, After inserting a probing cursor into the artery (on the screen), a signal indicating the blood flow velocity was produced. There was an audible and visual cue. The systolic and diastolic blood flow are represented by the peaks and ebbs in the signal. The spectrum k15] is made up of the peaks and ebbs [17].

Every asymptomatic adult or hypertensive patient at moderate risk for cardiovascular disease should have their intima-media thickness (IMT), a measure of subclinical atherosclerosis (asymptomatic organ damage), assessed. Values of intima-media thickness exceeding the 75th percentile (ASE) or 0.9 mm (ESC) ought to be regarded as abnormal.

Two of the three criteria—abnormal wall thickness (defined as C-IMT >1.5mm), abnormal shape (protrusion into the lumen, loss of alignment with adjacent artery wall border), and abnormal wall texture (brighter echoes than adjacent boundaries)—were used to determine whether plaque was present or not [18].

Statistical analysis: IBM SPSS, Chicago, USA's SPSS statistics software package, version 22.0, was used to conduct statistical analyses. Categorical variables were reported as frequencies and percentages, while continuous data were given as the mean with the standard deviation. The chi-squared test or Fisher's exact test was used to analyse categorical variables, while the Mann-Whitney U test and Student's t-test was used to analyse continuous variables in order to compare the characteristics of the two groups.

Results

In this study, 100 T2DM patients were involved; 88 of the patients were female and 12 were male, with a mean age of 52.4 ± 8.8 (F:M 7:1). The gender (five males and forty-five females; F:M 9:1; p=0.7) and mean age (54.1-20.3) of the 50 controls ranged from 33 to 70 years (p=0.2). The mean duration of this disease in T2DM group was 8 years (mean± SD= 8.5± 7.1 years). When compared to the control group, the T2DM group has significantly greater FPG, 2h-PG, HbA1c, TC, LDL, TG, and reduced HDL. The CIMT measurements showed a statistically significant difference between the T2DM patients and the controls, with higher values (mean 0.1 ± 0.02, p value 0.00) (Table 1).

Table 1. *Clinical and biochemical characteristics together with CIMT measurements of T2DM group and control group*

Characteristics	T2DM	Controls	P Value
	n = 100	n = 50	
Age (years)			
Range	33 — 70	33 — 70	
Mean ± SD	52.4 ± 8.8	54.1 ± 9.3	0.268
Gender			
Male	12 (12 %)	5 (10 %)	0.716
Female	88 (88 %)	45 (90 %)	
WC (cm)			
Range	78 — 138	77 — 138	
Mean ± SD	108.5 ± 9.9	105.6 ± 12.1	0.112
BMI			
Range	21.3 — 55	22.9 — 52	
Mean ± SD	36.7 ± 6.8	35.2 ± 6.6	0.193
HTN	31 (31 %)		
Duration of Disease (years)			
Range	1 — 35		
Mean ± SD	8.5 ± 7.1		
DM Treatment			
Insulin	40 (40 %)		
Oral hypoglycemic	37 (37 %)		
Sulphonylureas	30		
DPP4i	7		
Insulin + oral hypoglycemic	23 (23 %)		
Insulin+DPP4i	7		
Insulin+sulphonylureas	16		
FPG (mg/dL)			
Range	65 — 359	61 — 114	
Mean ± SD	174.5 ± 60.9	85.5 ± 9.6	**<0.001**
2h-PG (mg/dL)			
Range	110 — 649	90 — 161	
Mean ± SD	290.9 ± 99.4	120.4 ± 14.6	**<0.001**
HbA1c (%)			
Range	5.1 — 12.3	4.9 — 6.4	
Mean ± SD	8.7 ± 1.3	5.8 ± 0.5	**<0.001**
TC (mg/dL)			
Range	102 — 493	112 — 199	
Mean ± SD	230.7 ± 70.6	159.9 ± 30.2	**<0.001**
HDL (mg/dL)			
Range	26 — 69	40 — 60	
Mean ± SD	42.9 ± 9.9	51.4 ± 5.9	**<0.001**
LDL (mg/dL)			
Range	20 — 423	20 — 129	
Mean ± SD	140.9 ± 65.7	96.4 ± 22.4	**<0.001**
TG (mg/dL)			
Range	68 — 764	54 — 149	
Mean ± SD	213.3 ± 130.1	99.2 ± 25.1	**0.003**
Lt. CIMT (cm)			
Range	**0.06 — 0.3**	**0.05 — 0.12**	
Mean ± SD	0.1 ± 0.03	0.08 ± 0.02	**<0.001**
Rt. CIMT (cm)			
Range	0.07 — 0.2	0.06 — 0.12	
Mean ± SD	**0.1 ± 0.02**	**0.08 ± 0.01**	**<0.001**
Atherosclerosis	70 (70 %)	16 (32 %)	**<0.001**

Note. WC — Waist circumference, BMI — Body Mass Index, HTN — hypertension, DM — diabetic mellitus, FPG — Fast-post glucose, 2h-PG — Postprandial plasma glucose at two hours, TC — total cholesterol, HBA1c — haemoglobin A1c, HDL and LDL — High and Low densiy Lipoproteins, TG — Triglycerides, CIMT — The thickness of the carotid intima media, Rt — right, Lt — left, DPP4i — dipeptidyl peptidase 4 inhibitors

When comparing the T2DM group to the control group, there is a statistically significant increase in the incidence of aberrant capillary morphology, scarring, capillary haemorrhage, and sparse capillaries (p values <0.001, 0.001, 0.005, and 0.03 correspondingly). They also had higher frequency of Branched capillaries, extravasation of capillaries, crossed capillaries, corkscrew shape capillaries, reduced capillary length, enlarged Loops when compared with control group, but these changes didn't reach statistical significance (p value 0.17, 0.553, 0.551, 0.551, 0.454, 0.719 respectively). In T2DM group, there was 19 % of patients had scarring in the capillary field, 14 % had capillary hemorrhage, and 10 % had scanty capillaries and all these findings did not present at all in control group, so it cannot be considered as a normal variant. The control group has a significantly higher frequency of Modified NVC score ≤ 1 when compared with T2DM group. (Table 2, Figure 1).

There was no discernible variation in CIMT and NVC measures, morphological alterations, or statistical significance between the HbA1c ≥ 7 and the HbA1c < 7 groups in individuals with T2DM, but we noticed reduced NVC measurements in the HbA1c ≥ 7 group when compared with the HbA1c < 7 group in patients with T2DM. They have also higher frequency of scarring, capillary hemorrhage, scanty capillaries, large capillaries, branched capillaries, extravasation of capillaries and crossed capillaries when compared with the other group (Table 3).

The dyslipidemic group had a significantly higher FPG, 2h-PG, and HbA1c when compared with the non dyslipidemic group. There was a significantly higher frequency of HbA1c < 7 in the non dyslipidemic group when compared with dyslipidemic group, but no statistically significant difference between dyslipidemic and the non dyslipidemic group in patients with T2DM regarding CIMT measurements (Table 4).

Table 2. *NVC measurements of T2DM group and Control group*

	T2DM group	Control group	P value
Arterial Limb (μm)			
Range	4 — 13.9	4.1 — 16.1	
Mean ± SD	9.67 ± 2.72	9.04 ± 2.54	0.186
Venous Limb (μm)			
Range	10.4 — 19.2	8.06 — 24	
Mean ± SD	15.52 ± 2.57	14.39 ± 3.34	0.087
Capillary Loop (μm)			
Range	9.2 — 27.9	6 — 30.2	
Mean ± SD	18.58 ± 5.44	18.01 ± 5.17	0.582
Capillary Length (μm)			
Range	95.7 — 283.3	78.7 — 284.4	
Mean ± SD	162.69 ± 49.4	156.93 ± 44.1	0.619

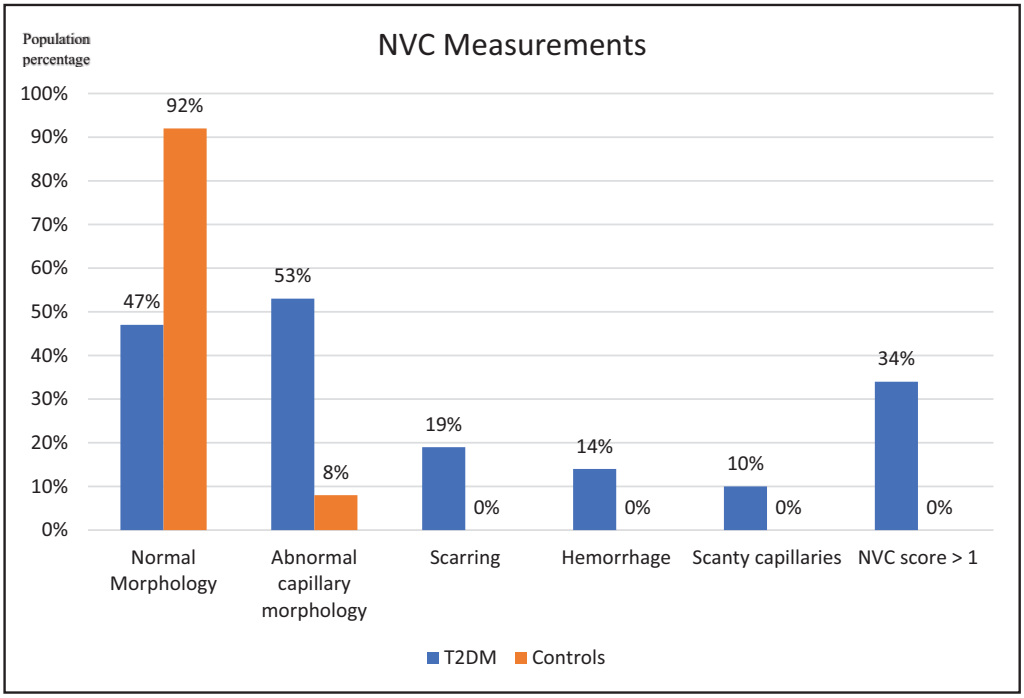

Figure 1. *Shows frequency of NVC measurements in T2DM group and control group*

Table 3. *Comparison of CIMT and NVC measurements between the HBA1c < 7 and HBA1c ≥ 7 groups in T2DM group*

Characteristics	HbA1c < 7	HbA1c ≥ 7	P Value
	n = 15	n = 85	
Lt. CIMT (cm)			
Range	0.07 — 0.13	0.06 — 0.3	
Mean ± SD	0.1 ± 0.02	0.1 ± 0.03	0.224
Rt. CIMT (cm)			
Range	0.08 — 0.13	0.07 — 0.2	
Mean ± SD	0.09 ± 0.03	0.1 ± 0.02	0.517
Atherosclerosis (%)	11 (73.3 %)	59 (69.4 %)	1.000

Note. HbA1c — hemoglobin A1c, CIMT — carotid intima media thichness, Rt — right, Lt — left

Table 4. *Comparison of clinical and biochemical characteristics between the dyslipidemic and non-dyslipidemic groups in T2DM group*

Characteristics	Dyslipidemic	Non-dyslipidemic	P Value
	n = 91	n = 9	
Age (years)			
Range	33 — 70	54 — 62	
Mean ± SD	51.9 ± 8.9	57.8 ± 3.6	**0.029**
Gender			
Male	11 (12.1 %)	1 (11.1 %)	1.000
Female	80 (87.9 %)	8 (88.9 %)	
WC (cm)			
Range	78 — 138	91 — 122	
Mean ± SD	108.5 ± 10.1	108.9 ± 8.8	0.838
BMI			
Range	21.3 — 55	28.3 — 51.2	
Mean ± SD	36.5 ± 6.7	39.2 ± 7.2	0.268
HTN	28 (30.8 %)	3 (33.3 %)	1.000
Duration of Disease (years)			
Range	1 — 35	1 — 20	
Mean ± SD	8.5 ± 7.1	9 ± 6.8	0.740
DM Treatment			
Insulin	34 (37.4 %)	6 (66.7 %)	0.151
Oral hypoglycemic	35 (38.5 %)	2 (22.2 %)	0.478
Insulin + oral hypoglycemic	22 (24.2 %)	1 (11.1 %)	0.680
FPG (mg/dL)			
Range	65 — 359	90 — 178	
Mean ± SD	178.7 ± 61.5	132.7 ± 34.5	**0.019**
2h-PG (mg/dL)			
Range	110 — 649	140 — 290	
Mean ± SD	298.9 ± 99.3	210.2 ± 56.1	**0.005**
HbA1c (%)			
Range	5.1 — 12.3	6.2 — 10.7	
Mean ± SD	8.8 ± 1.2	7.7 ± 1.6	**0.038**
HbA1c			
< 7	10 (11 %)	5 (55.6 %)	**0.003**
≥ 7	81 (89 %)	4 (44.4 %)	
Lt. CIMT (cm)			
Range	0.06 — 0.3	0.07 — 0.12	
Mean ± SD	0.1 ± 0.03	0.1 ± 0.02	0.513
Rt. CIMT (cm)			
Range	0.07 — 0.2	0.08 — 0.13	
Mean ± SD	0.1 ± 0.02	0.1 ± 0.02	0.815
Atherosclerosis	64 (70.3 %)	6 (66.7 %)	1.000

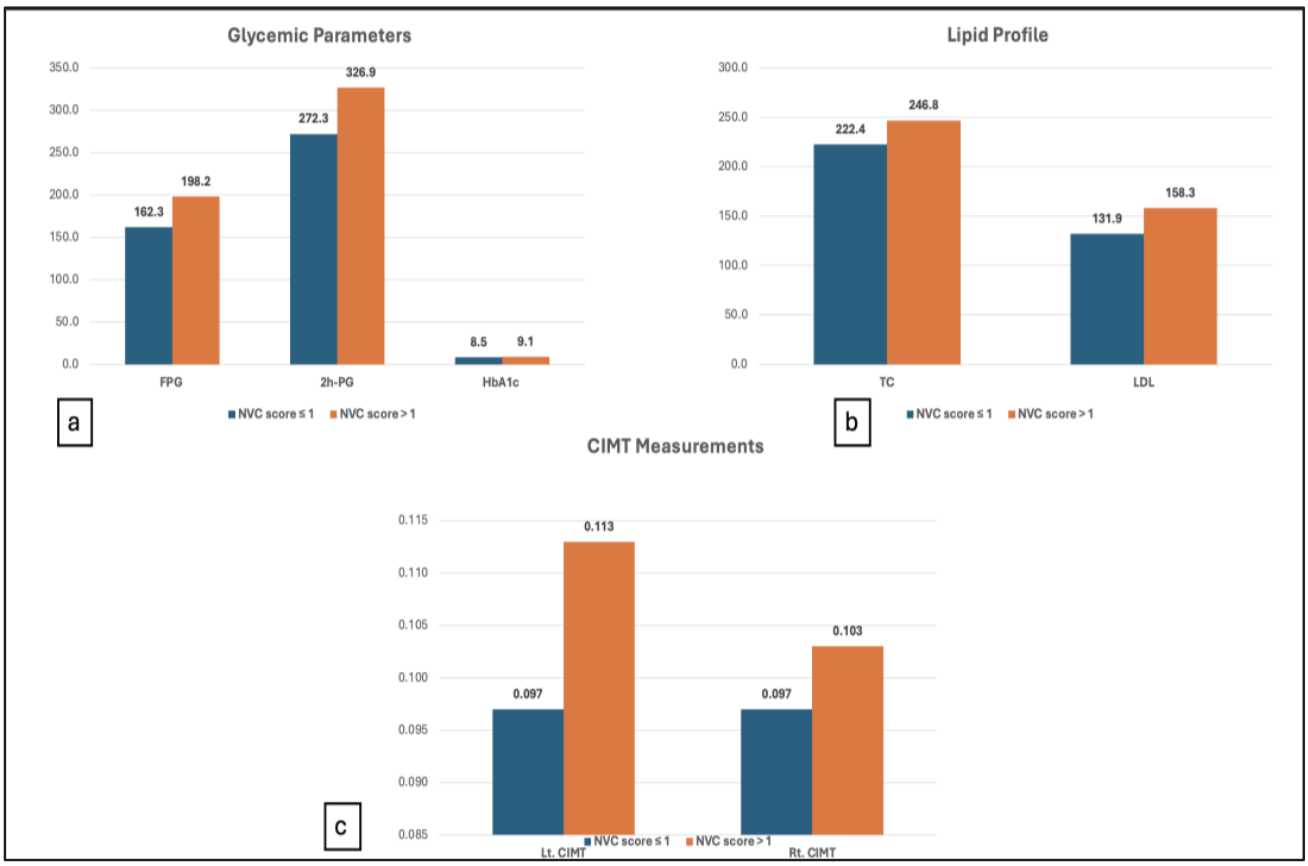

Figure 2. *Comparison between modified NVC score ≤ 1 and modified NVC >1 groups in T2DM patients as regards glycemic parameters (a), lipid profile (b) and right and left CIMT measurements(c)*

When comparing the group of type 2 diabetics with a Modified NVC score of ≤1 and those with Modified NVC score>1, FPG, 2h-PG, HbA1c, TC, and LDL all showed statistically significant increases, Lt and Rt CIMT measurements all showed statistically significant increases in the diabetics group with a Modified NVC score of >1 (figure 2).

Lt. CIMT was found to have a statistically significant direct association with age, TC, and LDL. Table 5 indicates that there was no statistically significant link discovered between CIMT measures and NVC measurements, although there was a statistically significant direct correlation between Rt. CIMT and age.

Discussion

Atherosclerosis in diabetic patients is a complex process which is the result of interaction of many factors, not only dyslipidemia, and total cholesterol and LDL are the most important parameters that affect vascular affection. The development of atherosclerosis was substantially correlated with increasing age. Thus, the ageing process that leads to atherosclerosis happens over a long period of time.

In our study we found that Rt. and Lt. CIMT were significantly higher in T2DM patients when compared to controls and this emphasize the hypothesis of premature atherosclerosis that occurs in diabetic patients despite that both groups had visceral obesity.

This is in line with the findings of Brohall G et al., who discovered that patients with diabetes mellitus had noticeably higher CIMT levels than healthy persons [19].

In our study there was a significantly abnormal capillary morphology in diabetic patients compared to controls in the form of capillary hemorrhage, scarring and branched capillaries, crossed capillaries, corkscrew shape capillaries, scanty capillaries and extravasation. In addition, type 2 diabetics had significantly higher frequencies of reduced capillary length and enlarged loops than the control group. There is also a significantly higher frequency of Modified NVC score ≤ 1 in controls when compared with T2DM group which signifies microvascular affection in diabetic patients, also we raise the hypothesis that the three morphological changes which are scarring, scanty capillaries and capillary hemorrhage never to be considered as normal variants as their incidence in controls is 0 %.

This is in line with the findings of Po-Chi Hsu et al., who reported that, in comparison to the controls patients, those with pre-DM or T2DM had considerably greater rates of microcirculation abnormalities and altered NVC scores [20].

Table 5. *Correlations between CIMT measurements with clinical and biochemical characteristics and NVC measurements.*

Characteristics		Lt. CIMT	Rt. CIMT
Age	R	0.302	0.286
	p value	**0.002**	**0.004**
W.C.	R	-0.053	0.120
	p value	0.601	0.236
BMI	R	-0.102	0.036
	p value	0.314	0.719
Duration of Disease	R	0.052	0.172
	p value	0.610	0.086
FPG	R	-0.033	-0.051
	p value	0.742	0.615
2hr-PG	R	-0.018	0.051
	p value	0.856	0.616
HbA1c	R	-0.019	0.006
	p value	0.849	0.953
TC	R	0.284	0.103
	p value	**0.004**	0.309
HDL	R	0.029	-0.044
	p value	0.776	0.665
LDL	R	0.253	0.077
	p value	**0.011**	0.447
TG	R	-0.057	-0.043
	p value	0.575	0.669
Arterial Limb	R	**-0.175**	**-0.154**
	p value	0.081	0.127
Venous Limb	R	0.028ab	0.065
	p value	0.779	0.521
Capillary Loop	R	-0.135	0.015
	p value	0.181	0.882
Capillary Length	R	-0.018	0.094
	p value	0.862	0.350

There was no significant difference regarding the CIMT measurements of patients with type 2 DM according to HbA1c level (HbA1c≥7 % and HbA1c<7 %). This may highlight that the process of atherosclerosis is an ongoing process which is not only related to disease control, but it may be related to other parameters in diabetic patients which need further studies.

High HbA1c and elevated CIMT were reported to be significantly correlated in the diabetic investigations by Mukai N et al. [21- 25], However, HbA1c and CIMT results [25] did not significantly correlate in research by Du HW et al. on diabetic mellitus patients.

This difference in results may be due to other factors that affect atherosclerosis like age, hyperlipidemia, duration of disease or even genetic factors. Study size and ultrasound method also may have a role e.g.: our study size is 150 subjects, while Mukai N et al have 2702 subjects the definition of carotid wall thickening also may vary e.g.: in our study it was defined as a maximum IMT of > 0.09 cm, while Mukai N et al defined it as a maximum IMT of >1.0 mm [19]. This also may be due to level of FPG, 2h PP in our patients who were not controlled even in those with HbA1c < 7 %.

The NVC characteristics of patients with type 2 diabetes mellitus were not significantly different between groups based on HbA1c level (HbA1c ≥ 7 % and HbA1c < 7 %). However, we did find that subjects with HbA1c ≥ 7 % had a higher frequency of Modified NVC score > 1, as well as scarring, capillary haemorrhage, large, branched, crossed capillaries, and extravasation.

Nevertheless, upon conducting a quantitative and qualitative analysis of the nailfold capillary abnormalities in type 2 diabetics and classifying them based on Modified NVC score (NVC ≤ 1 and NVC >1), we discovered that the diabetics with Modified NVC score >1 exhibited significantly higher levels of FPG, 2hr-PG, and HbA1c.

These findings suggested that in T2DM patients, alterations of the nailfold capillaries have been linked to poor glycaemic control.This may raise the hypothesis that tight glycemic control will affect microvascular changes before it conducts an effect on macrovascular changes and the first abnormality is the capillary morphology, this may explain that macrovascular complications will take more time to improve than microvascular complications and need more time of tight blood sugar control and may be the etiology of the absence of difference in intima media thickness between controlled and non-controlled patients.

According to Po-Chi Hsu et al, patients with HbA1c≥7 % showed a significantly higher NVC score. Subjects with HbA1c ≥ 7 % [21] had higher rates of shortened capillary length, irregular capillary distribution, abnormal capillary morphology, expanded loop, and abnormal flux.

In our study, no significant difference regarding to The CIMT was found in patients with type 2 DM classified according to lipid abnormalities and this highlights the hypothesis that atherosclerosis in diabetic patients is related not only to dyslipidemia but also to underlying process of inflammation and advanced glycation.

However, Lt. CIMT was proven to have a significant direct correlation with TC and LDL .This finding confirm that the process of atherosclerosis in diabetic patients is complex, and it is due to an interaction of many factors, one of them is dyslipidemia and mainly affected by TC and LDL and that the protective effect of HDL is not enough to protect our diabetic patients from the process of atherosclerosis, which needs a multidisciplinary approach to control it.

We found variables like age, FPG, 2hr-PG and HbA1c significantly higher in dyslipidemic than non dyslipidemic group.

Age, the length of diabetes, systolic and diastolic blood pressure, total cholesterol, triglycerides, LDL cholesterol, FPG, 2-hour postprandial glucose, and HbA1c were among the factors that Sunil et al. showed to have a significant and positive correlation with CIMT, while HDL cholesterol showed a negative correlation with the latter [26].

However, when we categorized type 2 diabetics according to Modified NVC score (NVC ≤ 1 and NVC >1), we found significantly higher levels of TC and LDL in the group of diabetics with Modified NVC score >1. This indicated that nailfold capillary abnormalities have been associated with dyslipidemia (mainly abnormalities in TC and LDL).

There were significantly higher Rt. CIMT and Lt. CIMT in the group of diabetics with Modified NVC score > 1 and so higher frequency of atherosclerosis in this group, so macrovascular abnormalities are more frequent in patients with abnormal capillaroscopic findings, and this means that microvascular and macrovascular complications are correlated to each other and that microvascular affection precedes macrovascular affection.

All these findings emphasize the importance of microvascular study in diabetic patients which may be an early detector for glycemic control in those patients and it is an easy non-invasive method to detect early changes in microvascular affection and an indicator for macrovascular affection.

Conclusion

We emphasized that glycated hemoglobin level affects earlier microvascular complications ealier than macrovascular complications which take longer duration.

Our study also emphasized the hypothesis of premature atherosclerosis among subjects with T2DM and revealed that NVC identified high frequencies of microvascular abnormalities among those patients. This study highlights the importance of microvascular study as well as macrovascular study.

We recommend that all patients with T2DM should undergo carotid doppler ultra-sonography to detect macrovascular affection as a routine investigation. Nailfold capillaroscopy should be done at earlier stage of diabetes mellitus to detect microvascular affection and for proper control of the disease to improve outcomes and avoid related complications.

Вклад авторов:

Все авторы внесли существенный вклад в подготовку работы, прочли и одобрили финальную версию статьи перед публикацией

Инасс Шалтоут: Анализ и проверка данных

Мэри Уади: Проведение капилляроскопии и проверка рукописи

Мазен Аттиа: Интерпретация данных

Ая Кхафаги: Сбор данных

Сара А. Хассан: Проверка результатов, написание рукописи

Author Contribution:

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

Inass Shaltouta: Analyzing and revising the data

Mary Wadieb: Doing the capilloroscopy and revising the manuscript

Mazen Attiac: Interpretting data

Aya Khafagyd: Collecting data

Sarah A. Hassanc: Revising the results, Writing the manuscript

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НЕИНВАЗИВНЫЕ ПРЕДИКТОРЫ ВЫРАЖЕННОЙ ГИСТОЛОГИЧЕСКОЙ АКТИВНОСТИ ПРИ ХРОНИЧЕСКИХ ЗАБОЛЕВАНИЯХ ПЕЧЕНИ: РОЛЬ МАТРИКСНЫХ МЕТАЛЛОПРОТЕИНАЗ

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A Noninvasive Predictors of Significant Histological Activity in Chronic Liver Diseases: The Role of Matrix Metalloproteinases

Резюме

Цель исследования: изучение прогностической значимости клинико-лабораторных маркеров печеночной патологии, в том числе компонентов системы матриксных металлопротеиназ (ММП), для выявления умеренной/выраженной активности при хронических заболеваниях печени (ХЗП). **Материалы и методы.** Обследовано 76 пациентов ХЗП вирусной или алкогольной этиологии в возрасте от 18 до 64 лет. Минимальная (индекс гистологической активности — ИГА 1-3 балла), слабовыраженная (ИГА 4-8 баллов), умеренная (ИГА 9-12 баллов) и выраженная морфологическая активность (ИГА более 12 баллов) выявлялись в 19 (25,0 %), 34 (44,7 %), 14 (18,4 %) и 9 (11,9 %) случаев соответственно. Методом иммуноферментного анализа определяли содержание в крови ММП-1, ММП-9, тканевого ингибитора матриксных металлопротеиназ-1 (ТИМП-1), рассчитывали соотношение ТИМП-1/ММП-1, ТИМП-1/ММП-9. **Результаты.** По данным многофакторной логистической регрессии, умеренная/выраженная гистологическая активность ХЗП была ассоциирована с показателями γ-глютамилтранспептидазы (ГГТ) (отношение шансов (ОШ) 1,016; 95 % доверительный интервал (ДИ) (1,006-1,024), p=0,001), международного нормализованного отношения (МНО) (ОШ 1,079; 95 % ДИ (1,028-1,132), p=0,002), соотношения ТИМП-1/ММП-9 (ОШ 0,554; 95 % ДИ (0,380-0,809), p=0,002). Комбинация этих параметров имела чувствительность 82,6 %, специфичность 92,5 % и точность 89,5 % в выявлении ИГА 9 и более баллов. **Заключение.** Увеличенные значения ГГТ и МНО, а также сниженное соотношение ТИМП-1/ММП-9 являются независимыми факторами риска умеренной/выраженной гистологической активности при ХЗП, что обусловлено их участием в процессах печеночного воспаления.

Ключевые слова: *хронические заболевания печени, гистологическая активность, матриксная металлопротеиназа-9, тканевой ингибитор матриксных металлопротеиназ-1, γ-глютамилтранспептидаза, международное нормализованное отношение*

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Abstract

Aim of investigation. To study the prognostic significance of clinical and laboratory markers of liver pathology, including components of the matrix metalloproteinase (MMP) system, to identify moderate/significant activity in chronic liver diseases (CLD). **Materials and methods.** 76 patients with CLD of viral or alcoholic etiology aged from 18 to 64 years were examined. Minimal (histological activity index — HAI 1-3 points), minor (HAI 4-8 points), moderate (HAI 9-12 points) and significant morphological activity (HAI more than 12 points) were detected in 19 (25.0 %), 34 (44.7 %), 14 (18.4 %) and 9 (11.9 %) of cases, respectively. Enzyme immunoassay was used to determine the blood levels of MMP-1, MMP-9, tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), and the of TIMP-1/MMP-1, TIMP-1/MMP-9 was calculated. **Results.** According to multivariate logistic regression data, moderate/significant histological activity of CLD was associated with γ-glutamyltranspeptidase (GGT) (odds ratio (OR) 1.016; 95 % confidence interval (CI) (1.006-1.024), p=0.001), international normalized ratio (INR) (OR 1.079; 95 % CI (1.028-1.132), p=0.002), and TIMP-1/MMP-9 ratio (OR 0.554; 95 % CI (0.380-0.809), p=0.002). The combination of these parameters had sensitivity of 82.6 %, specificity of 92.5 % and accuracy of 89.5 % in detecting HAI of 9 or more points. **Conclusion.** The increased values of GGT and INR, as well as a reduced ratio of TIMP-1/MMP-9, are independent risk factors for moderate/significant histological activity in CLD, due to their participation in the processes of hepatic inflammation.

Key words: *chronic liver diseases, histological activity, matrix metalloproteinase-9, tissue inhibitor of matrix metalloproteinases-1, γ-glutamyltranspeptidase, international normalized ratio*

Conflict of interests

Co-author of the article Yagoda A.V. is a member of the editorial board of the journal «The Russian Archives of Internal Medicine». The article passed the journal's peer review procedure. Yagoda A.V. was not involved in the decision to publish this article. The authors did not declare any other conflicts of interest

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ALT — alanine aminotransferase, AST — aspartate aminotransferase, GGT — γ-glutamyl transpeptidase, CI — confidence interval, HAI — histological activity index, INR — international normalised ratio, MMP — matrix metalloproteinase, OR — odds ratio, MPTI — matrix metalloproteinase tissue inhibitor, CLD — chronic liver diseases, Ac — accuracy, AUC — area under ROC-curve, HBV — hepatitis B virus, HCV — hepatitis C virus, HDV — hepatitis D virus, MCV — mean corpuscular volume, NPV — negative predictive value, PPV — positive predictive value, RDW — red blood cell distribution width, Se — sensitivity, Sp — specificity

Introduction

Chronic liver diseases (CLD) constitute a serious health-related issue, which is associated with high prevalence, morbidity, and mortality, low quality of life, enhanced patient disability, and increased organ transplant requirements. CLDs are mainly represented by infections associated with hepatitis B (HBV) (29 %) and C (HCV) (9 %) viruses, alcoholic (2 %) and non-alcoholic (59–60 %) fatty liver disease; autoimmune, hereditary, and drug-induced lesions (1 %) are rare [1].

Despite the vaccination and use of nucleos(t)ide analogues, chronic HBV infection is diagnosed in 240–296 million people, which is more commonly diagnosed in China (29 %), India (6.6 %), and Nigeria (5.8 % of cases). 1.5 million new HBV infection cases are diagnosed annually; every year, 800–820 thousand people die from its complications (liver cirrhosis and hepatocellular carcinoma) [1, 2, 3].

Approximately 58–113 million people globally are infected with HCV (0.8–1.1 %); 1.5–1.8 million new cases are detected annually, which exceeds the number of deceased and recovered patients. Half of all infection cases are reported in China, Pakistan, India, Egypt, Russia, and the USA [1, 2].

12 million patients with the HDV infection have been diagnosed globally; anti-HDV antibodies are detected in 4.5–16.4 % of HBsAg-positive persons. Mongolia is the first in the list of HBsAg-positive and HDV-infected population (36.9 %), followed by Guinea-Bissau (23.9 %), Gabon (22 %), Mauritania (19.4 %), Togo (18.5 %), and Moldova (15 %) [4].

The exact prevalence of alcoholic liver disease is unknown, while its trend can be followed with the alcohol consumption per capita. Alcohol consumption leads to 3.3 million deaths annually, 5.3–10 % of premature deaths among people of working age [5]; alcohol is associated with a high risk of mortality from liver diseases (27 %) and hepatocellular carcinoma (20–30 %) [1, 2].

Determining the severity of necrotic & inflammatory changes in the liver is important to optimize the patient management from the point of prognosis and timely therapeutic decisions. Transcutaneous liver biopsy followed

by the morphological study is a golden standard of evaluating the hepatic inflammation severity. However, biopsy is an invasive diagnostic method, which is not available in the limited resource setting, but rather associated with the low patient compliance, unfavorable risks, variability of results, insufficient representativeness, and impossibility of monitoring changes [6].

Currently almost no non-invasive markers and scales exist that help to diagnose hepatic inflammation, especially in patients with stably normal aminotransferase activity. Actitest (BioPredictive, France) including six parameters (alanine aminotransferase, bilirubin, γ-glutamyl transpeptidase, haptoglobin, alpha-2 macroglobulin, apolipoprotein A1) is positioned as a non-invasive tool in the diagnosis of hepatic disease activity. Along with that, some data demonstrate the insufficient Actitest concordance with morphological signs of chronic hepatitis [7, 8], while several parameters of the scale are not used routinely, which limits its use.

Besides controlling the accumulation and degradation of the extracellular matrix components, matrix metalloproteinases (MMP) and their tissue inhibitors (MPTI) actively participate in the inflammatory process, angiogenesis, and hepatic regeneration [9]. MMPs affect the replication, penetration, and spread of hepatotropic viruses, release the membrane-bound pro-inflammatory cytokines, destroying the basal membrane markers, and promote white blood cell transfer to tissues [9, 10]. Increased MMP-2 and MMP-9 expression is associated with white blood cell extravasation and infiltration, enhanced inflammation in the setting of the ischemic-reperfusion liver injury [11]. MMP-8 deficiency in the acute hepatitis model impaired white blood cell migration and chemokine release, which can prove the role of this matrix metalloproteinase in the regulation of inflammation [9].

However, the association of serum MMP and MPTI levels and the histological activity of chronic liver diseases has not been always detected [12, 13, 14]. TIMP-1, MMP-3 parameters have been more commonly used in the non-invasive diagnosis of fibrosis [6], while the prognostic ability of metalloproteinases and their inhibitors regarding the severity of inflammation in patients with hepatic diseases have not been analyzed at all.

The study is aimed at analyzing the diagnostic significance of clinical & laboratory parameters, including the components of the matrix metalloproteinase system, in the prediction of moderate/significant morphological activity in chronic liver diseases.

Materials and Methods

A total of 76 patients with chronic liver diseases (27 females, 49 males) aged 18 to 64 years (41 (31; 48) years). Inclusion criteria: histologically confirmed CLDs of viral or alcoholic origin; age over 18 years; signed informed consent to participate in the study, including the liver biopsy. Exclusion criteria: liver diseases of any other etiology; acute and chronic (exacerbated) clinically significant somatic diseases; drug abuse; psychic diseases; pregnancy and lactation; malignancies.

Study design: open-label cross-sectional study.

Clinical characteristics of patients with CLDs are presented in Table 1.

Chronic hepatitis was detected in 59 (77.6 %) cases (HBV — 16, HCV — 30, HDV — 13), liver cirrhosis (Child-Pugh Class A) — in 17 (22.4 %) patients: that of viral etiology — in 13 (17.1 %) patients (HBV — 2, HCV — 8, HDV — 3), alcoholic cirrhosis — in 4 (5.3 %) cases.

The viral etiology of hepatic diseases was established based on the presence of HBsAg and HBV DNA (HBV infection), anti-HCV antibodies and HCV RNA (HCV infection), anti-HDV antibodies and HDV RNA (HDV infection). The alcoholic etiology of hepatic diseases was diagnosed based on the history, AUDIT test (> 8 points), detection of alcoholic stigmata and laboratory markers (AST elevation > ALT, increased mean corpuscular volume (MCV), γ-glutamyl transpeptidase activity).

Depending on the ALT and/or AST parameters, biochemical CLD activity was divided into minimal (increase < 3 x upper limit of normal (ULN)), moderate (3–5 fold increase), and severe (> 5 x ULN), which were observed in 57 (75.0 %), 11 (14.5 %), and 8 (10.5 %) patients, respectively.

Mesenchymal-inflammatory syndrome was diagnosed based on increased erythrocyte sedimentation rate (ESR), C-reactive protein, a- and g-globulin, fibrinogen levels; its manifestations were detected in 19 (25 %) patients. Cholestatic syndrome detected in 7 (9.2 %) cases was determined with the increased alkaline phosphatase, γ-glutamyl transpeptidase, and conjugated bilirubin levels.

To evaluate the histological activity, all patients underwent the transcutaneous liver biopsy in the morning in the fasting condition under ultrasound guidance with the 18G needle, obtaining the liver sample 1.5 mm wide and ≥ 1.5 cm long (the sample should have contained at least six portal tracts). Biopsy specimens were fixed in formalin, embedded in paraffin, and stained with hematoxyline-eosin. All liver specimens were analyzed by the pathologist not knowing the clinical patient characteristics.

Based on the histological activity index (HAI) (R.J. Knodell et al., 1981), the morphological activity was stratified into minimal (1–3 points), mild (4–8 points), moderate (9–12 points), and severe (> 12 points), which were detected in 19 (25.0 %), 34 (44.7 %), 14 (18.4 %), and 9 (11.9 %) cases, respectively. Liver fibrosis (V. Desmet et al.) F0, F1, F2, F3, F4 was detected in 8 (10.5 %), 20 (26.3 %), 18 (23.7 %), 13 (17.1 %), 17 (22.4 %) cases, respectively.

The gender- and age-matched control group consisted of 72 almost healthy persons.

Table 1. *Clinical characteristics of patients with CLD*

Parameters	Patients with CLD, n=76
Gender (women/men, n (%))	27 (35,5) / 49 (64,5)
Age (years)	41,0 (31,0; 48,0)
AST (u/l) (reference values: men 0-40 u/l, women 0-31 u/l)	43,0 (25,9; 81,0)
ALT (u/l) (reference values: men 0-41 u/l, women 0-32 u/l)	56,5 (28,0; 99,9)
GGT (u/l) (reference values: men 8-61 u/l, women 7-32 u/l)	51,0 (33,5; 95,5)
Alkaline phosphatase (u/l) (reference values: men 40-130 u/l, women 36-106 u/l)	111,0 (70,0; 210,0)
Total bilirubin (μmol/l) (reference values: 0-17 μmol/l)	15,9 (11,9; 25,45)
Conjugated bilirubin (μmol/l) (reference values: 0-5 μmol/l)	3,7 (3,0; 7,1)
ESR (mm/h) (reference values: men 2-10 mm/h, women 2-15 mm/h)	8,0 (5,0; 12,0)
C-reactive protein (mg/l) (reference values: 0-5 mg/l)	0,7 (0,2; 4,4)
Fibrinogen (g/l) (reference values: 2.2-3.97 g/l)	2,63 (2,23; 3,55)
Albumin (g/l) (reference values: 34-48 g/l)	45,0 (42,0; 47,0)
Prothrombin time (sec) (reference values: 9.1-12.1 sec)	16,19 (12,65; 17,45)
Prothrombin index (%) (reference values: 90-105 %)	91,0 (88,0; 97,5)
INR (reference values: 0.85-1.15)	1,13 (1,02; 1,2)
Total cholesterol (mmol/l) (reference values: 0-5.17 mmol/l)	4,28 (3,75; 4,65)
Platelets (x10^9/l) (reference values: 150-400x10^9/l)	187,5 (150,5; 238)
Moderate/severe biochemical activity n (%)	19 (25,0)
Mesenchymal inflammatory syndrome n (%)	19 (25,0)
Cholestatic syndrome n (%)	7 (9,2)

Footnote: quantitative data are presented as Me (Q1; Q3), categorical data as n (%)
Abbreviations: ALT — alanine aminotransferase, AST — aspartic aminotransferase, CLD — chronic liver diseases, GGT — γ-glutamyltranspeptidase, ESR — erythrocyte sedimentation rate, INR — international normalized ratio

The study was approved by the Local Ethics Committee of the University (Protocol No. 100 dated 17/06/2020).

The immunoenzymatic method was used to detect the blood levels of MMP-1 (RayBiotech, USA), MMP-9 (Bender MedSystems GmbH, Austria), MPTI-1 (Aviscera Bioscience, USA), with subsequent calculation of MPTI-1/MMP-1 and MPTI-1/MMP-9 ratios.

The results were statistically processed (StatTech v. 3.1.7; StatTech LLC, Russia). Quantitative values with the distribution other than normal are presented as medians (Me) and the interquartile range (Q1; Q3). Differences were detected using the Mann-Whitney test. Categorical data presented as percentages (%) were evaluated using the χ^2 test with Yates' correction for continuity. The Spearman rank correlation coefficient, odds ratio (OR), and its 95 % confidence interval (CI) were calculated. The following was calculated: sensitivity, specificity, positive and negative predictive value, accuracy. The association of independent variables and the dependent variable (HAI ≥ 9 points) was analyzed using the logistic regression analysis method. ROC analysis and model informativity were evaluated using the area under ROC curve. Differences were considered statistically significant with $p < 0.05$.

Results

Patients with chronic liver diseases compared to the control group had MPTI-1 (565 (478; 691) ng/mL and 387.5 (284.5; 482.0) ng/mL, $p < 0.00001$) and MMP-1 (22 (12.75; 33.63) ng/mL and 4.95 (2.64; 14.25) ng/mL, $p < 0.00001$) in blood; MPTI-1/MMP-9 (3.02 (1.3; 6.7) U and 1.40 (0.95; 2.05) U, $p = 0.00002$) were higher, while plasma MMP-9 levels (188.0 (95.15; 407.0) ng/mL and 320.0 (200.0; 362.0) ng/mL, $p = 0.056$) and MPTI-1/MMP-1 (23.95 (15.0; 53.15) U and 67.90 (24.10; 139.85) U, $p < 0.00001$) were lower than in healthy persons. The parameters analyzed did not depend on the sex and age of persons in the study.

Changes in the matrix metalloproteinase system parameters were unidirectional for the alcoholic and viral CLD etiology, without statistically significant differences.

Maximum MPTI-1, MPTI-1/MMP-1 and MPTI-1/MMP-9 ratios, and minimum MMP-1 values were reported in the patient group with the F4 fibrosis vs. the F0-F3 fibrosis.

Blood MMP and MPTI levels in patients with CLD were not associated with hepatitis B/C virus replication, besides the direct correlation of serum MMP-9 values with the HBV (r = 0.65; p = 0.004) and HCV (r = 0.39; p = 0.02) viremia level. Histological activity index parameters in CLD did not correlate with the HBV (r = 0.22; p > 0.05) and HCV (r = 0.15; p > 0.05) viremia level.

Higher serum levels of aspartate (AST) and alanine (ALT) aminotransferases, gamma-glutamyl transpeptidase (GGT), total bilirubin, higher ESR, international normalised ratio (INR) levels, lower platelet count were observed with the moderate and severe histological activity of liver diseases compared to the minimal one; moderate/high cytolysis and mesenchymal inflammation syndromes were also more common among the former ones. In this patient group, blood MPTI-1 and MMP-9 levels were higher, while the MPTI-1/MMP-9 ratio was lower than in minimum morphological activity cases (Table 2).

Table 2. *The relationship of markers of liver pathology and components of the matrix metalloproteinase system with HAI*

Parameters	Patients with CLD, n=76		P Value
	HAI <9 points, n=53	HAI ≥9 points, n=23	
Gender (women/men, n (%))	17 (32,1) / 36 (67,9)	10 (43,5) / 13 (56,5)	p>0,05
Age (years)	40,0 (29,5; 44,0)	45,0 (35,0; 47,0)	p>0,05
AST (u/l) (reference values: men 0-40 u/l, women 0-31 u/l)	39,9 (22,5; 55,5)	67,2 (28,0; 95,0)	p=0,016
ALT (u/l) (reference values: men 0-41 u/l, women 0-32 u/l)	49,0 (25,35; 66,95)	97,4 (39,0; 113,0)	p=0,007
GGT (u/l) (reference values: men 8-61 u/l, women 7-32 u/l)	37,0 (21,5; 60,0)	91,0 (55,0; 107,2)	p<0,001
Alkaline phosphatase (u/l) (reference values: men 40-130 u/l, women 36-106 u/l)	90,0 (68,5; 163,0)	210,0 (70,0; 210,0)	p=0,061
Total bilirubin (μmol/l) (reference values: 0-17 μmol/l)	15,0 (11,15; 18,5)	21,9 (14,0; 34,2)	p=0,003
Conjugated bilirubin (μmol/l) (reference values: 0-5 μmol/l)	4,0 (3,0; 5,8)	3,0 (1,7; 5,0)	p>0,05
ESR (mm/h) (reference values: men 2-10 mm/h, women 2-15 mm/h)	5,0 (4,5; 12,0)	9,0 (8,0; 9,0)	p=0,013
C-reactive protein (mg/l) (reference values: 0-5 mg/l)	0,6 (0,2; 2,4)	1,03 (0,2; 4,8)	p>0,05
Fibrinogen (g/l) (reference values: 2.2-3.97 g/l)	2,6 (2,29; 3,5)	3,0 (1,7; 5,0)	p>0,05
Albumin (g/l) (reference values: 34-48 g/l)	45,0 (42,0; 47,0)	43 (40,5; 47,0)	p>0,05
Prothrombin time (sec) (reference values: 9.1-12.1 sec)	15,9 (12,35; 17,0)	16,5 (13,0; 17,2)	p>0,05
Prothrombin index (%) (reference values: 90-105 %)	91,0 (88,0; 97,5)	91,0 (79,0; 95,0)	p>0,05
INR (reference values: 0.85-1.15)	1,1 (1,0; 1,15)	1,17 (1,11; 1,3)	p<0,001
Total cholesterol (mmol/l) (reference values: 0-5.17 mmol/l)	4,28 (3,75; 4,59)	4,28 (3,75; 4,54)	p>0,05
Platelets (x10^9/l) (reference values: 150-400x10^9/l)	210 (143; 238)	161 (104; 172)	p=0,008
Moderate/severe biochemical activity n (%)	9 (17,0)	10 (43,5)	p=0,031
Mesenchymal inflammatory syndrome n (%)	9 (17,0)	10 (43,5)	p=0,031
Cholestatic syndrome n (%)	3 (5,7)	4 (17,4)	p>0,05
TIMP-1 (ng/ml)	528,0 (429,0; 621,0)	664,0 (564,0; 713,0)	p<0,001
MMP-1 (ng/ml)	21,0 (13,88; 30,6)	25,3 (10,1; 31,35)	p>0,05
MMP-9 (ng/ml)	119,0 (73,65; 254,0)	576,0 (200,0; 790,0)	p<0,001
TIMP-1/MMP-1	22,36 (14,89; 35,95)	25,4 (15,71; 36,4)	p>0,05
TIMP-1/MMP-9	3,5 (1,9; 6,8)	1,2 (0,6; 2,8)	p<0,001

Footnote: quantitative data are presented as Me (Q1; Q3), categorical data as n (%); criterion Yates's chi-squared test, Mann-Whitney criterion
Abbreviations: ALT — alanine aminotransferase, AST — aspartic aminotransferase, CLD — chronic liver diseases, GGT — γ-glutamyltranspeptidase, ESR — erythrocyte sedimentation rate, HAI — histological activity index, INR — international normalized ratio, MMP — matrix metalloproteinase, TIMP — tissue inhibitor of matrix metalloproteinases

Increased risk (IHA ≥ 9 points) was associated with the following parameters: ESR ≥8 mm/h, GGT ≥ 53.8 U/L, INR ≥ 1.11, total bilirubin ≥ 20.5 μmol/L, ALT ≥ 70.5 U/L, alkaline phosphatase ≥ 189 U/L, AST ≥ 53 U/L, platelet count ≤ 187x10^9/L, as well as with moderate/high biochemical activity, mesenchymal-inflammatory syndrome. Blood MPTI-1 levels ≥ 554 ng/mL, MMP-9 levels ≥ 410 ng/mL, and MPTI-1/MMP-9 ratio ≤ 1.59 U were also associated with the increased risk of significant inflammation. The most optimal area under curve was detected in cases of MMP-9 levels ≥ 410 ng/mL (0.82±0.05), GGT ≥ 53.8 U/L (0.81±0.05), MPTI-1 ≥ 554 ng/mL (0.74±0.06), MPTI-1/MMP-9 ≤ 1.59 U (0.74±0.06), INR ≥ 1.11 (0.73±0.06). Sensitivity and specificity values for the aforementioned parameters were as follows: MMP-9 — 60.9 % and 92.5 %, GGT — 91.3 % and 69.8 %, MPTI-1 — 87.0 % and 58.5 %, MPTI-1/MMP-9 — 60.9 % and 83.0 %, INR — 87.0 % and 54.7 %, respectively (Table 3).

The multivariate regression analysis was arranged to detect the most significant factors associated with moderate and high histological activity (HAI ≥ 9 points). It included 13 factors (AST, ALT, GGT, total bilirubin, alkaline phosphatase, ESR, INR values, platelet count, moderate/high biochemical activity, mesenchymal inflammatory syndrome, blood MMP-9 and MPTI-1 levels, MPTI-1/MMP-9 ratio) which were associated with a high risk of severe morphological hepatic changes based on the univariate analysis.

According to the multivariate analysis results, association with HAI ≥ 9 points was detected for parameters of the MPTI-1/MMP-9 ratio (OR 0.554; 95 % CI (0.380–0.809), p = 0.002), GGT (OR 1.016; 95 % CI (1.006–1.024), p = 0.001), INR (OR 1.079; 95 % CI (1.028–1.132), p = 0.002). The odds of moderate/severe histological activity decreased 1.805-fold with MPTI-1/MMP-9 increase by 1 U and increased 1.016-fold with GGT increase by 1 U/L, 1.079-fold with INR increase by 1.

The association observed was described by the following equation:

$$z = -9.077 - 0.590 \times X_{\text{MPTI-1/MMP-9}} + \\ + 0.015 \times X_{\text{GGT}} + 7.599 \times X_{\text{INR}},$$

where z is the value of the logistic regression function; $X_{\text{MPTI-1/MMP-9}}$ is a value of the MPTI-1/MMP-9 ratio (U); X_{GGT} is a value of the GGT activity (U/L); X_{INR} is the INR value; –9.077 is a regression constant; –0.590; 0.015; 7.599 are regression coefficients for corresponding variables.

Table 3. *Diagnostic significance of liver pathology markers and components of the matrix metalloproteinase system in the detection of HAI ≥9 points*

Parameters	OR (95 % CI)	AUC (M±SE)	Se (%)	Sp (%)	PPV (%)	NPV (%)	Ac (%)
AST ≥53.0 u/l	4,8 (1,7-13,5) p=0,0035	0,65±0,07 p=0,033	65,2	71,7	50,0	82,6	69,7
ALT ≥70.5 u/l	6,4 (2,2-18,7) p=0,0007	0,68±0,07 p=0,015	65,2	77,4	55,6	83,7	73,7
GGT ≥53.80 u/l	24,3 (5,1-116,1) p=0,0001	0,81±0,05 p<0,001	91,3	69,8	56,8	94,9	76,3
Alkaline phosphatase ≥189 u/l	5,3 (1,8-15,3) p=0,0019	0,64±0,07 p=0,049	60,9	77,4	53,8	82,0	72,4
Total bilirubin ≥20.5 μmol/l	7,2 (2,4-21,2) p=0,0004	0,70±0,06 p=0,007	65,2	79,2	57,7	84,0	75,0
ESR ≥8 mm/h	61,0 (3,5-1057,0) p=0,0047	0,66±0,07 p=0,026	100	56,6	50,0	100	69,7
INR ≥1.11	8,1 (2,1-30,4) p=0,0021	0,73±0,06 p=0,002	87,0	54,7	45,5	90,6	64,5
Platelets ≤187x10^9/l	4,3 (1,5-12,7) p=0,008	0,67±0,07 p=0,016	73,9	60,4	44,7	84,2	64,5
Modeate/severe biochemical activity	3,8 (1,3-11,2) p=0,0175	0,62±0,07 p=0,017	43,5	83,0	52,6	77,2	71,1
Mesenchymal inflammatory syndrome	3,8 (1,3-11,2) p=0,0175	0,62±0,07 p=0,017	43,5	83,0	52,6	77,2	71,1
TIMP-1 ≥554 ng/ml	9,39 (2,48-35,55) p=0,001	0,74±0,06 p=0,001	87,0	58,5	47,6	91,2	67,1
MMP-9 ≥410 ng/ml	19,06 (5,1-71,27) p=0,001	0,82±0,05 p<0,001	60,9	92,5	77,8	84,5	82,9
TIMP-1/MMP-9 ≤1,59	7,6 (2,53-22,9) p=0,0003	0,74±0,06 p<0,001	60,9	83,0	60,9	83,0	76,3

Abbreviations: Ac — accuracy, ALT — alanine aminotransferase, AST — aspartic aminotransferase, AUC — area under the ROC curve, CI — confidence interval, GGT — γ-glutamyltranspeptidase, ESR — erythrocyte sedimentation rate, HAI — histological activity index, INR — international normalized ratio, MMP — matrix metalloproteinase, NPV — negative predictive value, OR — odds ratio, PPV — positive predictive value, Se — sensitivity, Sp — specificity, TIMP — tissue inhibitor of matrix metalloproteinases

The probability of HAI ≥ 9 points is calculated using the formula: $p = 1 : (1 + e^{-z})$, where e is the base of the natural algorithm (2.72), p is the probability of HAI ≥ 9 points.

The p threshold in the cut off point was 0.457. HAI ≥ 9 points was predicted with p over or equal to this value. Parameters of the diagnostic significance for p ≥ 0.457 were as follows: sensitivity — 82.6 %, specificity — 92.5 %, positive predictive value — 82.6 %, negative predictive value — 92.5 %, accuracy — 89.5 %. The regression model was statistically significant ($p < 0.001$), with area under ROC-curve of 0.911±0.043 (95 % CI: 0.828–0.995), which proved an excellent model quality.

Discussion

Thus, chronic liver diseases are accompanied by increased blood MMP-1, MPTI-1 levels, and the MPTI-1/MMP-9 ratio, with decreased MPTI-1/MMP-1 levels. Based on our data, the matrix metalloproteinase system plays an important role in the development of necroinflammatory lesions in hepatic diseases. Thus, moderate and significant morphological activity of chronic liver diseases was associated not only with cytolysis, inflammation, and cholestasis markers, INR values, and platelet count, but also with increased serum MPTI-1, MMP-9 levels and decreased MPTI-1/MMP-9 ratio.

Older age, higher ALT, AST, GGT, alkaline phosphatase, prothrombin time, INR values, lower albumin levels and platelet count were typical for patients with chronic viral hepatitis and significant inflammation that was confirmed morphologically [15]. Severe hepatic inflammation during the HBV infection was associated with the increased rate of the male sex, HBeAg positivity, increased total bilirubin, ALT, AST, alkaline phosphatase, GGT, prothrombin time, INR, viremia, mean platelet volume and mean corpuscular volume vales, as well as with low albumin levels, platelet and white blood cell counts [16, 17, 18, 19]. It is presumed that the Golgi 73 protein, antibodies to the core hepatitis B antigen, globulins, and red blood cell distribution width (RDW) are associated with the histological activity of chronic liver diseases; however, their predictive values have not been validated [19–22].

Aminotransferase parameters are widely used in routine practice for the indirect evaluation of hepatic inflammation in chronic hepatitis; however, their correlation with the process activity is limited by the effects of various factors [16, 23]. It is considered that AST better predicts inflammatory changes in the liver (due to slower excretion and mitochondrial damage associated with severe inflammation), but ideal values for the cutoff point have not been defined. Besides, the predictive aminotransferase value is negatively affected by periodic fluctuations of their levels.

The association of platelets with lesion and inflammation severity in liver diseases is based on the participation in white blood cell recruitment and accumulation (CD8+ T-cells) in parenchyma; interaction with Kupfer cells mediated by the von Willebrand factor-GP1b complex; ability to preserve viruses from degradation or, vice versa, to present them to immune cells; sinusoidal blood flow modulation due to the secreted serotonin [24].

MMP-1 plays a role in the regression of inflammation, fibrosis, and liver regeneration, cleaving collagen and proteoglycans, interleukin-1β, and tumor necrosis factor-α, activating MMP-2 and -9, participating in the white blood cell migration, facilitating the molecule release from storage pools. In liver diseases, MMP-1 is expressed predominantly by stellate and inflammatory cells [9, 25, 26]. Blood MMP-1 levels increased in chronic hepatitis C [27]; however, some data demonstrate its generally decreased levels in CLD [13], including in the setting of viral, alcoholic hepatitis, and cirrhosis, as well as in late stages of the primary biliary cholangitis [28]. One cannot exclude the presence of normal serum MMP-1 levels in alcoholic and non-alcoholic fatty liver disease, early stages of primary biliary cholangitis [28, 29].

MMP-9 is secreted by endotheliocytes, Kupfer cells, white blood cells, and macrophages [28, 30]. It destroys type IV collagen, elastin, fibronectin, increases the permeability and white blood cell extravasation and infiltration, promotes inflammation, impairs liver regeneration [30]. MMP-9 expression and activity increased with ischemic-reperfusion damage, chronic hepatitis C, alcoholic liver [9, 27]. Blood MMP-9 levels increased in acute alcoholic intoxication [31], chronic hepatitis B [32]. In other studies serum MMP-9 levels decreased in viral or alcoholic hepatitis and cirrhosis, primary biliary cholangitis [28], but were normal in alcoholic or non-alcoholic fatty liver disease [28, 29].

Several studies demonstrated increased serum levels of several matrix metalloproteinases in liver diseases associated with the increased histological activity [12, 14]. Blood MMP-7 levels or its expression in the biliary epithelium directly correlated with morphological signs of inflammation in primary sclerosing cholangitis and biliary atresia [14]. MPTIs expressed by stellate cells and macrophages also negatively affect the inflammation severity [33]. Thus, MPTI-1 hyperexpression is an indicator of stellate cell activity, which is associated with necroinflammatory changes in the liver [34]. With that, one cannot exclude negative correlation or the absence of association of serum matrix metalloproteinase values and their inhibitors with the hepatic inflammation severity. Blood MMP-1 levels in children or adults with liver diseases did not depend on the morphological activity degree [12, 13].

Currently, non-invasive markers reflecting the intensity of hepatic inflammation are almost lacking. The multivariate regression analysis has demonstrated that increased GGT activity, INR values, and decreased MPTI-1/MMP-9 ratio had independent effects on the development of moderate and severe histological activity (HAI ≥ 9 points).

Prior logistic regression data established that combinations of GGT and prothrombin time with the alkaline phosphatase activity [15], ALT and HBV viremia levels [17] or AST and anti-HB core levels [16] were independent predictors of significant inflammation in chronic viral hepatitis B. Prognostic models developed based on these parameters evaluated the hepatic inflammation (≥ G2) with moderate sensitivity (61.0–80.8 %) and specificity (60.8–84.2 %) (area under ROC curve 0.714–0.767) [15, 16, 17]. Besides ALT, AST, and GGT activity, platelet count and HBsAg levels in blood [18], RDW, platelet count, and albumin levels [19] had significant effects on inflammation severity in HBV infection. Based on the multivariate regression analysis, necroinflammation predictors in autoimmune hepatitis included Golgi 73 protein and GGT levels in blood, while in patient with primary biliary cholangitis those included serum Golgi 73 protein, alkaline phosphatase, IgM levels, and the platelet count [21].

Being a microsomal enzyme of hepatic ductal and canalicular epithelium, GGT controls the metabolism of glutathione, the main antioxidant molecule in cells. In this aspect, the association of GGT with the inflammation severity is caused by the modulating enzyme effects on the pro-oxidant activity and endothelial dysfunction [35], which plays some role in hepatic injury and inflammation [36, 37]. It is proposed that unlike ALT, GGT is a more sensitive marker of necroinflammatory changes in chronic liver diseases [16].

The association of increased INR levels with the CLD activity detected in our study is caused the ability of inflammation and cellular necrosis to cause the activation and consumption of blood coagulation factors. On the other hand, activated coagulation proteases may modulate the inflammatory activity with receptors on mononuclear or endothelial cells, on platelets, altering the production of pro-inflammatory cytokines, adhesins, or causing the apoptosis of inflammatory cells. Besides, the prolonged prothrombin time (and INR, respectively) reflects the impaired synthetic hepatocyte function worsened by the severity of inflammatory hepatic lesions [16].

The detected predictive significance of decreased MPTI-1/MMP-9 ratio (with MMP-9 prevailing over MPTI-1) regarding the histological activity is based on the ability of MMP-9 produced by immune cells (among others) to impair regeneration processes, enhance the parenchymatous inflammation due to the activation of pro-inflammatory cytokines and enhanced white blood cell migration. It is presumed that MMP-9 reflects the inflammatory process in the liver more than fibrogenesis [28]. Thus, in ischemic-reperfusion liver injury MMP-9 provided extravasal white blood cell migration and promoted inflammation [11].

Thus, the imbalance in the matrix metalloproteinase system is associated with the morphological activity in chronic liver diseases in the form of MMP-9 hyperproduction associated with enhanced inflammation severity. The inclusion of risk factors (GGT, INR, MPTI-1/MMP-9) into a simple mathematical model facilitates the personified prediction of moderate/severe activity in patients with chronic liver diseases.

Conclusion

The study has demonstrated that increased GGT and INR parameters, as well as decreased MPTI-1/MMP-9 ratio values are independent predictors of moderate/severe inflammation in chronic liver diseases. Their association with the inflammation severity is related to the effects on endothelial dysfunction, activity of proinflammatory cytokines, migration of immune cells into the hepatic parenchyma. The combination of GGT, INR, and MPTI-1/MMP-9 is of high significance in the diagnosis of histological activity index ≥ 9 points; thus, it can be used in chronic liver diseases as a non-invasive marker of significant inflammation.

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Ягода А.В.: концепция и дизайн исследования, научное консультирование, редактирование рукописи, утверждение окончательного варианта статьи

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Author Contribution:

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

Yagoda A.V.: the concept and design of the study, scientific advice, editing the article, approval of the final version of the manuscript

Koroy P.V.: the concept and design of the study, writing of the manuscript, editing the article, collection and processing of material, search for literary sources, analysis and systematization of literature data, approval of the final version of the manuscript

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ЛЕТАЛЬНЫЙ КЛИНИЧЕСКИЙ СЛУЧАЙ АМИЛОИДНОЙ КАРДИОМИОПАТИИ У ПОЖИЛОЙ ПАЦИЕНТКИ

O.V. Soldatova, I.Y. Goryanskaya

Order of the Red Banner of Labor Medical Institute named after. S.I. Georgievsky, Crimean Federal University named after V.I. Vernadsky", Department of Internal Medicine № 1, Simferopol, Russia

Amyloid Cardiomyopathy: Review of A Fatal Case Report

Резюме

Пристальное внимание к проблеме амилоидной кардиомиопатии в последние годы обусловлено значительным приростом выявляемости заболевания на фоне повышения чувствительности и специфичности методов визуализации, применяемых в кардиологической практике, наряду с появлением новых перспективных методов диагностики и специфической терапии Выбор тактики лечения системного амилоидоза напрямую зависит от результатов типирования амилоидогенных белков, которое стало возможным благодаря развитию протеомики, основанной на масс-спектрометрии. На сегодняшний день доказано, что важной и часто недиагностированной причиной хронической сердечной недостаточности и нарушений сердечного ритма, особенно в пожилом возрасте, является амилоидная кардиомиопатия. Существует более 15 типов белков-предшественников, способных вызывать системный амилоидоз, однако только 2 из них накапливаются в интерстиции сердца: легкие цепи клонального иммуноглобулина (AL) и тетрамерный белок транстиретин (TTR). О значительной распространенности генетического транстиретинового амилоидоза дикого типа (ATTRwt), ранее именуемого старческим системным амилоидозом, говорят следующие цифры: у 13 % пациентов, госпитализированных по поводу декомпенсации хронической сердечной недостаточности с сохранной фракцией выброса левого желудочка диагностической находкой явилась транстиретиновая амилоидная кардиомиопатия, среди пациентов старше 80 лет данная патология post mortem выявляется в 20-25 % патологоанатомических заключений, и в 37 % случаев в группе долгожителей (пациентов старше 97 лет). Даже при ранней диагностике ATTR-амилоидоза продолжительность жизни от момента появления первых симптомов составляет 10-12 лет, так как заболевание необратимо прогрессирует, приводит к инвалидности вследствие тяжелого поражения сердца и полинейропатии. Поздняя же диагностика системного амилоидоза обусловлена низкой осведомленностью врачей первичного звена, наличием коморбидности у пожилых пациентов, отсутствием специфических симптомов заболевания и доступных диагностических скрининг-методов, и предопределяет неблагоприятный прогноз данного заболевания, особенно при формировании амилоидной кардиомиопатии.

Нами представлено описание клинического случая пожилой пациентки с торпидным течением прогрессирующей декомпенсированной застойной сердечной недостаточности, окончившейся летально на 3-и сутки госпитализации. Прижизненная верификация транстиретиновой амилоидоидной кардиомиопатии не представлялась возможной. Эхокардиографические критерии приблизили нас к диагнозу, а патологоанатомические исследования позволили подтвердить диагноз системного амилоидоза с преимущественным поражением сердца.

Ключевые слова: *системный амилоидоз, амилоидная кардиомиопатия, сердечная недостаточность, сердечно-сосудистые заболевания, клинический случай*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

Источники финансирования

Авторы заявляют об отсутствии финансирования при проведении исследования

Соответствие принципам этики

Информированное согласие не требуется в силу невозможности идентифицировать пациента

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Abstract

Close attention to the problem of amyloid cardiomyopathy in recent years has been caused by a significant increase in the disease detection simultaneously with increased sensitivity and specificity of imaging methods used in cardiological practice, along with the emergence of new promising diagnostic methods and specific therapy. The choice of treatment tactics for systemic amyloidosis directly depends on the results of typing of amyloidogenic proteins, which became possible due to the development of proteomics based on mass spectrometry. To date, it has been proven that amyloid cardiomyopathy is an important and often undiagnosed cause of chronic heart failure and cardiac arrhythmias, especially in the elderly. There are more than 15 types of precursor proteins capable of causing systemic amyloidosis, but only 2 of them accumulate in the interstitium of the heart: light chains of clonal immunoglobulin (AL) and tetrameric protein transthyretin (TTR). The significant prevalence of wild-type genetic transthyretin amyloidosis (ATTRwt), formerly referred to as senile systemic amyloidosis, is indicated by the following figures: in 13 % of patients hospitalized for decompensation of chronic heart failure with preserved left ventricular ejection fraction, transthyretin amyloid cardiomyopathy was a diagnostic finding, among patients over 80 years of age, this pathology is detected post mortem in 20-25 % of pathoanatomic reports, and in 37 % of cases in the long-lived group (patients over 97 years of age). Even with early diagnosis of ATTR-amyloidosis, the life expectancy from the moment the first symptoms appear is 10-12 years, as the disease progresses irreversibly, leading to disability due to severe heart damage and polyneuropathy. The late diagnosis of systemic amyloidosis is due to the low awareness of primary care physicians, the presence of comorbidity in elderly patients, the absence of specific symptoms of the disease and available diagnostic screening methods, and determines an unfavorable prognosis of this disease, especially with the formation of amyloid cardiomyopathy. The relevance of this topic is due to the need to improve diagnostic algorithms and reduce the time for primary diagnosis of amyloid cardiomyopathy in order to improve the prognosis of the disease.

We have described a clinical case of an elderly patient with a torpid course of progressive decompensation of congestive heart failure, which ended fatally on the 3rd day of hospitalization. Echocardiographic criteria brought us closer to the diagnosis of amyloid cardiomyopathy, but pathoanatomic studies have confirmed the diagnosis of systemic amyloidosis with predominant heart damage.

Key words: *systemic amyloidosis, amyloid cardiomyopathy, heart failure, cardiovascular diseases, clinical case*

Conflict of interests

The authors declare no conflict of interests

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Conformity with the principles of ethics

Informed consent is not required due to the impossibility of identifying the patient

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AL — amyloidosis light chain, ATTRwt — amyloidosis transtyretin wild type, NT-proBNP — N-terminal prohormone of brain natriuretic peptide, NYHA — New York Heart Association, SpO2 — peripheral oxygen saturation, TTR — transthyretin, AVO — atrioventricular opening, BP — blood pressure, AC — amyloid cardiomyopathy, BMI — body mass index, LVEDD — left ventricle end-diastolic dimension, LVEDV — left ventricular end-diastolic volume, COI — cutoff index, LVESD — left ventricle end-systolic dimension, chest CT — computed tomography of thoracic organs, LV — left ventricle, LA — left atrium, IVS — interventricular septum, IU — international units, MSCT — multispiral computed tomography, MRI — magnetic resonance tomography, ICU — intensive care unit, RV — right ventricle, RA — right atrium, PCR — polymerase chain reaction, PET — positron emission tomography, CF — cardiac failure, ESR — erythrocyte sedimentation rate, CRP — C-reactive protein, LVPWT — left ventricular posterior wall thickness, IVST — interventricular septum thickness, LVWT — left ventricular wall thickness, EF — ejection fraction, FC — functional class, daily monitoring of ECG — 24-hour Holter monitoring, CCFwLVEF — chronic heart failure with preserved left ventricle ejection fraction, CDH — central district hospital, RR — respiratory rate, HR — heart rate, ECG — electrocardiography, EMB — endomyocardial biopsy, ECA — cardiac electrical axis, echoCG — echocardiography

Introduction

Amyloidosis is a multisystem disease, associated with depositions of insoluble amyloid fibrils containing incorrectly aggregated proteins, in the extracellular space. Each type of amyloidosis has its own type of amyloidogenic precursor protein, the name of which underpins the modern classification of the disease [1]. It has been proven that amyloid cardiomyopathy (AC) significantly worsens disease prognosis, and in 98 % of cases it develops in primary systemic amyloidosis, such as AL-amyloidosis caused by deposition of immunoglobulin light chain (AL), and TTR-amyloidosis (ATTR), where transthyretin tetramers are deposited (ATTR) [2]. Interstitial infiltration of amyloid in the heart impacts restrictive cardiomyopathy phenotype on the pathogenic level and causes myocardial diastolic and (later) systolic dysfunction. Cardiac remodelling and amyloid deposition in the heart walls can lead to heart rhythm and conductivity disorders. In elderly patients, reduced quality of life can be a result not only of the age, but also of non-specific symptoms of AC due to ATTRwt, which vary from minimal to the symptoms of progressive decompensated cardiac failure (CF). Therefore, routine examination is not sufficient for diagnosis verification. The peculiarities of AC diagnosis lie in identification of suspicious clinical signs, associated with amyloidosis phenotype, and suspicion verification using imaging and specific disease-identifying laboratory tests [3]. Given the absence of specific signs and symptoms of this disease, as well as availability of sensitive diagnostic methods, such as heart MRI and scintigraphy, endomyocardial biopsy (EMB) with subsequent amyloidogenic

protein typing using immunohistological and biochemical assays, early diagnosis and timely initiation of disease-specific therapy is a topical problem in the real-time clinical practice. The incidence of AC, low rates of early diagnosis verification and, as a result, lack of ability to timely initiate disease-specific therapy, lay behind the importance of a detailed discussion of a lethal case study of amyloid cardiomyopathy in an elderly female patient.

Clinical case study

On 02/09/2024 *Patient M, a 75-year-old female patient,* was urgently admitted to the intensive care unit (ICU) of N. A. Semashko Republican Clinical Cardiological Dispensary in Simpheropol in serious condition; the severity of her condition was caused by cardiopulmonary failure and generalised oedema syndrome. Upon admission, the patient complained of marked weakness, heart problems, shortness of breath after minor physical activities (walking for 5–10 minutes), swollen ankles, abdomen dilatation. The patient noted weight loss of over 10 % over the past six months.

Past medical history: according to the patient, she got ill in February 2024, when she noted arrhythmia, but she did not seek medical advice. In June, after the patient returned from health resort treatment, her condition deteriorated: shortness of breath and lower limb swelling developed, and in June the patient was admitted to the internal medicine ward at the central district hospital at the place of the patient's residence. Chest CT dated 30/07/2024 showed right pneumohydrothorax, compensated right atelectasis, cardiomegaly, pulmonary engorgement, ascites. Pleural puncture was performed: pleural fluid examination showed serous transudate with the relative density of 1,010 g/mL, protein: 19 g/L; microscopic findings: mesothelium — 5–6 per HPF, lymphocytes — 3–4 per HPF, RBC — 10–12 per HPF, no atypical cells. She received symptomatic treatment and was discharged without any improvements. Because of persistent signs of congestive CF, one month later, the patient was hospitalised to the geriatric ward of the Crimean Republican Clinical Hospital for War Veterans with the following diagnosis: CCF, stage 2B; senile asthenia. EchoCG dated 07/08/2024 showed dilated right heart and left atrium, impaired diastolic myocardial function, EF 50 %; valves: unremarkable. Repeated chest CT dated 08/08/2024 confirmed cardiomegaly, bilateral hydrothorax and signs of pulmonary engorgement: right pleural space — free fluid (density: approx. 8 HU), layer thickness: up to 48 mm; left cavity: free fluid (density: approx. 5 HU), layer thickness: up 9 mm. Abdomen CT dated 08/08/2024: ascites, hydrosarca, fatty hepatosis.The therapy included treatment of CCF symptoms (furosemide 100 mg/day, verospiron 50 mg/day, metoprolol 50 mg/day, candesartan 32 mg/day), steroids (dexamethasone 8 mg/day), lipid-lowering drugs (rosuvastatin 5 mg/day). The patient was discharged without improvement. At the place of residence, the patient had follow-up chest X-ray examination (29/08/2024): the horizontal fluid level on the right is at the level of the IV rib, left pleural diaphragmal corner is free. Since there were no any positive changes in her condition, the patient had to visit the outpatient clinic at the cardiac dispensary, where she was ungently hospitalised.

Physical examination: severe general condition, lucid; body temperature: 36.4 °C, BMI = 18; by auscultation: above the lungs, the respiration is harsh, weaker in side areas, and absent in lower sections; no rales; respiratory rate is 24/min; oxygen saturation (SpO2) 96 %; muffled, arrhythmic cardiac tones, no heart murmurs; HR 60 bpm, arrhythmical pulse of poor volume, BP 85/55 mm Hg; symmetrical lower limb oedema up to the upper third of shin; abdomen soft on palpation, enlarged because free fluid, positive fluctuation symptom. Complete blood count showed no abnormal results: Hb 133 g/L, RBC 4.43×10^{12}/L, platelets 175×10^{9}/L, WBC 8.6×10^{9}/L, ESR 20 mm/h. Blood biochemistry: glucose 4.9 mmol/L, cholesterol 3.8 mmol/L, bilirubin 14.7 mmol/L, urea 23.0 mmol/L, creatinine 197 mmol/L, total protein 57 g/L, albumin 30 g/L, ALT 32.9 U, AST 24.7 U, potassium 4.9 mmol/L, sodium 129.3 mmol/L, calcium 1.27 mmol/L. Coagulation profile: prothrombin time 25.0, PTI 41.2 %, INR 2.0, fibrinogen A 4.5 g/L, APPT 41 s, thrombin clotting time 21 s. Cardiac markers: myoglobin — 72.0 ng/mL (normal range: < 70 ng/mL), troponin I — 0.14 ng/mL (normal range: < 0.01 ng/mL), NT-proBNP — 9,086 pg/mL. Markers of viral hepatites, HIV, syphilis: negative. Urinalysis results show proteinuria 0.12 g/L, leukocyturia — 10 per HPF. ECG upon admission (Figure 1): normal voltage; atrial flutter with AV conduction; HR 108 bpm, electrical cardiac axis is of normal position, left ventricle hypertrophy.

Transthoracic echoCG (Figure 2) dated 04/09/2024: LA 4.6 cm, LVEDD 3.6 cm, LVESD 3.0 cm, LVEDV index 46 mL/m^2, LVPWT 1.3 cm, IVST 1.3 cm, LVEF 36 %, RV 2.9 cm; moderately dilated right ventricle and both atria; normal LV dimension and volume; valves — unremarkable; moderate concentric LV myocardial hypertrophy; echo-structure of LV myocardium looks finely grained and shiny with typical glow; marked relative tricuspid insufficiency; moderate pulmonary hypertension with systolic pulmonary pressure of up to 42 mm Hg; significantly lower contractility of LV myocardium; significantly impaired diastolic function; right pleural cavity with signs of up to 600 mL of free fluid; no free fluid in pericardial cavity and left pleural cavity. Longitudinal deformity of the left ventricle was not accessed due to unavailability of technical capacities.

Clinical examination results were used to verify the primary diagnosis: primary amyloidosis with predominant cardiac involvement, complicated by stage 2B CCF with reduced myocardial contractility (EF 36 %), functional class 4 (NYHA); persistent atrial

fluttering (CHA2DS2-VASc 4 points, HAS-BLED 1 point, EHRA 2b); right-sided hydrothorax, ascites. The patient had therapy complying with clinical guidelines and medical assistance standards for her congestive heart failure, including cardiotropic (a combination of valsartan + sacubitril, bisoprolol), dehydration (furosemide, spironolactone), antiarrhythmic (amiodarone), anticoagulation (rivaroxaban) therapy and SGLT2 inhibitors (dapagliflozin) (standard doses). EMB was planned to histologically confirm the type of systemic amyloidosis.

Due to the antiarrhythmic therapy, on day 2 of hospitalisation the patient had sinus rhythm (Figure 3); however, the patient's condition remained the same (critical but stable). Despite the combined diuretic therapy (furosemide 100 mg intravenous drip and oral spironolactone 100 mg), no positive diuresis was observed, and hydrosarca persisted.

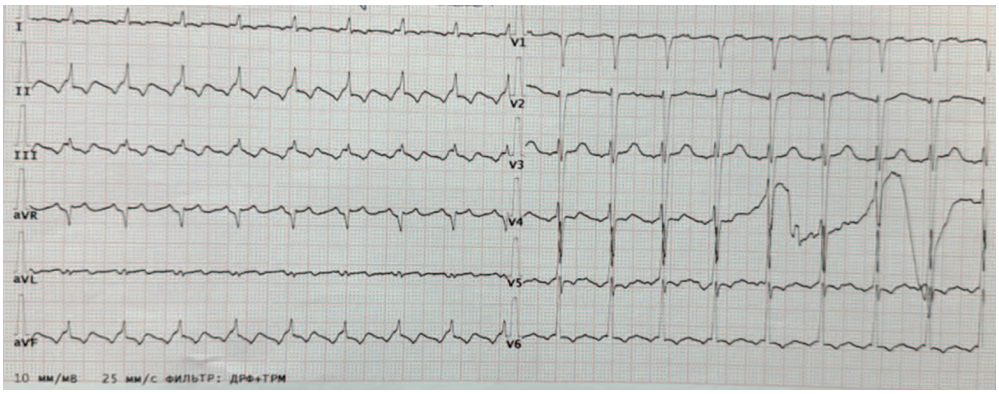

Figure 1. *ECG on admission — Atrial flutter*

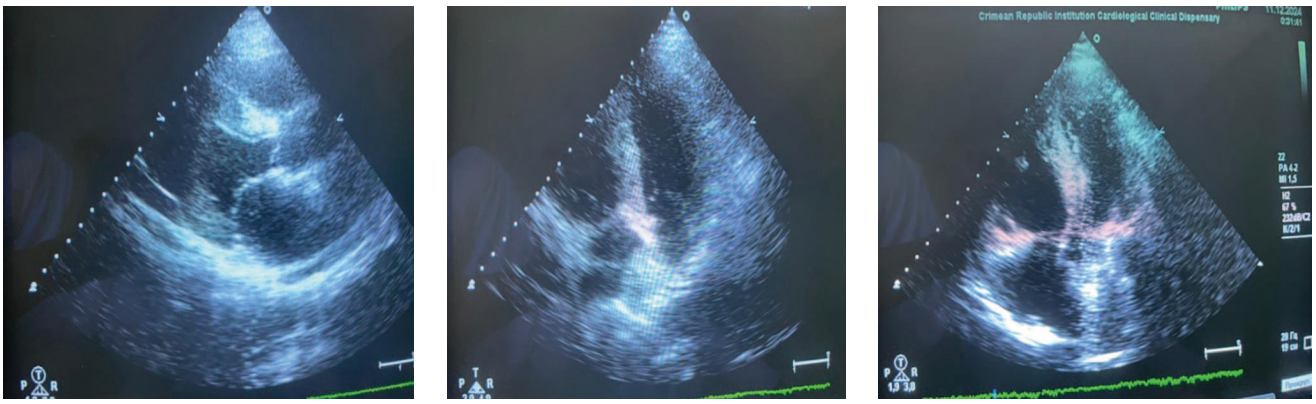

Рисунок 2. *Характерное для амилоидной кардиомиопатии «свечение» при трансторакальной ЭхоКГ*
Примечание. А — парастернальная позиция по длинной оси; Б и В — верхушечная позиция по длинной оси
Figure 2. *Amyloid cardiomyopathy "glow" during transthoracic echocardiography findings*
Note. A — parasternal position along the long axis; Б, B — apical position along the long axis

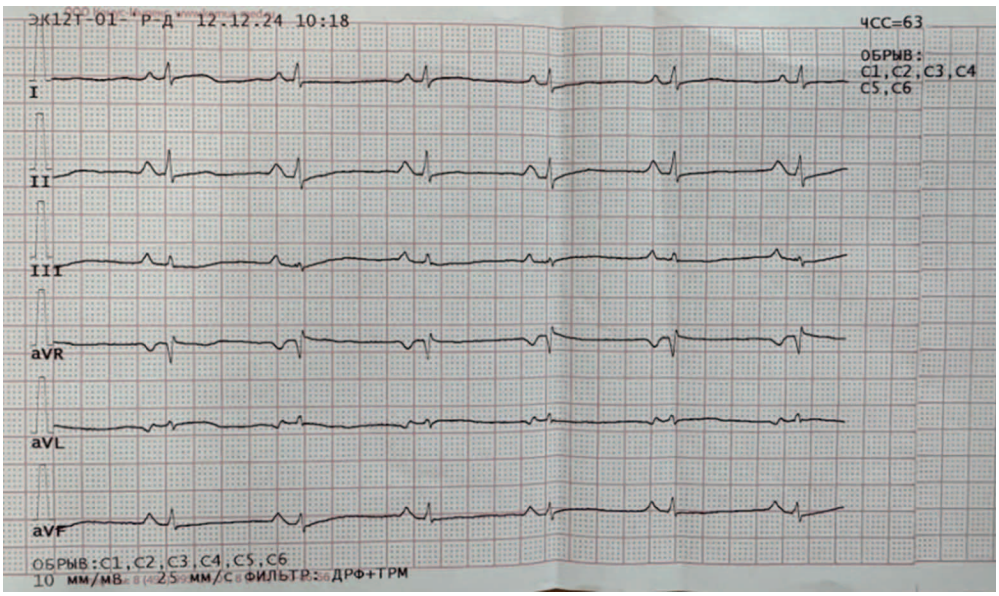

Figure 3. *ECG on the second day of hospitalization — sinus rhythm restoring after pharmacological cardioversion*

On day 3 of hospitalisation, the patient's condition deteriorated: she suddenly lost consciousness, unassisted breathing was ineffective, and resuscitation was initiated. Despite the intensive care, the patient went into cardiac arrest, monitors showed asystole, and the patient was pronounced clinically dead. Cardiac compressions, tracheal intubation, breathing support with a breathing bag and intravenous adrenaline were ineffective, and in 30 minutes, the natural death was pronounced.

Autopsy Results

A yellow clear fluid was found: in abdomen — 1,000 mL, in right pleural cavities — 2,000 mL, in left pleural cavities — 1,000 mL, in pericardial cavity — 100 mL. Heart: 700 g, 14×11×7 cm, with rounded apex, formed by LV; enlarged cardiac cavities, right atrioventricular opening (AVO) perimeter: 12 cm, left AVO: 10 cm, aorta: 7 cm. The myocardium is of cartilaginous density, light-brown in colour, shiny; LV wall thickness in the cross-section 1.0 cm from the fibrous atrioventricular ring 1.8 cm, RV — up to 0.5 cm. Coronary arteries are rigid, with single dense greyish-yellow plaques covering up to 50 % of intima surface, the opening is 50 % occluded. The lungs are pushed forwards by the fluid; dense, a lot of foamy liquid and thin blood drop from the cur surface.

Histological examination

Heart: positive staining with Congo red for amyloid and positive green glow in polarised light; uneven vascular congestion, thickening and amyloidosis of small and medium-sized vessel walls; perivascular, intermuscular foci of amorphous eosinophilic amyloid masses; thinning, atrophy and tortuosity of muscle fibres; dystrophy and moderate hypertrophy of cardiac myocytes; focal fragmentation of muscle fibres.

Brain: perivascular, pericellular oedema; glial cell dystrophy, areas of brain tissue rarefication; vascular congestion.

Lungs: poor blood supply to vessels; thickened interalveolar septa with eosinophilic amyloid deposits; the same deposits are observed along the vessels.

Liver: preserved frame structure; vast amyloid deposits in vessel walls, along portal tracts; dystrophic hepatic cells, oedema.

Kidneys: thickened, sclerotic glomerule capsule and vascular walls, amyloid deposits. Parenchyma with focal lymphocytic and globo-cellular infiltration, sclerosis and hyaline degeneration; vast areas of amyloid deposits along the vessels. Areas of amyloid deposits along the vessels are observed also in the pancreas.

The autopsy results show that the death was caused by progressive cardiopulmonary failure, namely pulmonary and brain oedema, resulting from the primary disease: primary systemic amyloidosis with predominant cardiac involvement.

Discussion

Numerous recent studies show undervaluation of ATTR amyloidosis as a cause of arrhythmias and impaired contractility, as well as progressive CF in elderly patients [4], which was observed in this clinical case study. The diagnostic search for the causes of torpid decompensated CCF and atrial fluttering was limited to ischaemic heart disease, acquired valvular problems and amyloid cardiomyopathy. The medical history of the patient did not show any data on arterial hypertension, chronic or acute forms of ischaemic heart disease, including myocardial infarction, or valvular heart disease, which could cause progressive CCF, that is why AC was most probably. According to the current idea, wild type ATTR (ATTR-wt) is associated with generalised amyloid deposits in interstitial tissue of parenchymal organs because of age-related defects of transthyretin tetramers secretion by the liver [5], and it can bring about the mentioned cardiac signs and symproms. Also, a typical clinical sign of AC was observed: reduced QRS voltage seen on ECG and mismatch between ECG results of myocardial hypertrophy and echoCG observations, i.e., reduced ECG/echoCG index below 7.8; sensitivity and accuracy of this sign are 94 % and 82 %, respectively, while its negative predictive value — 97 % [6]. A minor increase in cardiac markers (myoglobin — 72.0 ng/mL, troponin I — 0.14 ng/mL) ruled out massive myocardial damage, while an excessively high NT-proBNP level (9,086 pg/mL) suggested severe myocardial dysfunction from other causes. A combination of cardiac manifestations, such as weakness, arrhythmia, severe drug-resistant decompensated CCF (hydrosarca). ECG signs and echoCG observations: dilated right heart and LV, concentric LV myocardial hypertrophy (LV wall thickness up to 1.3 cm), diastolic and myocardial dysfunction (EF 36 %), and pathognomonic fine-grained structure of LV myocardium with glow confirmed AC. The only thing left to do was to identify the type of amyloidosis. The key differential diagnosis was to confirm ATTR- or AL type of systemic amyloidosis, which have similar manifestations, observed in this patient, namely she was over 70 years of age, lost weight and had symptoms of torpid progressive CCF. In this case study, where the patient did not have any extracardiac manifestations, the hypothesis of ATTRwt phenotype was supported only by arrhythmia (atrial flutter) [7]; studies show that the incidence atrial fibrillation/flutter in patients with AC in ATTR-wt was higher (71 % of patients) than in AL amyloidosis (26 %) [8]. Minimal proteinuria (0.12 g/L) did not help reliably differentiate between ATTR- and AL-amyloidosis, although, the latter is more often associated with nephrotic levels of proteinuria. Unfortunately, we did not have a chance to perform planned intravital identification of the type of systemic amyloidosis: subcutaneous tissue biopsy and EMB with subsequent identification of amyloid protein type, tests for plasma cell dyscrasia (bone marrow biopsy

with cytofluorometry, analysis of released paraproteins: immunofixation and electrophoresis of serum and urine proteins with quantification of monoclonal and polyclonal immunoglobulins). Even postmortem, it was impossible to verify systemic ATTR amyloidosis using routine autopsy methods, since no immunohistological and biochemical amyloid analysis was performed [9]. According to the literature, symptoms of CCF are a predictor of poor outcome in patients with systemic amyloidosis, because, if untreated, the mean survival rate is approximately one year, and half a year if symptoms of severe CCF are present [10]. This fact was confirmed in this clinical case study: six months after the onset of first cardiac symptoms (arrhythmia) and three months after CCF symptoms, the patient died of torpid CCF progression. Independent risk factors of poorer survival rates in AC from ATTR are also age, NYHA FC III–IV, systolic BP < 100 mm Hg, resistance to diuretics, while NT-proBNP levels ≥ 1,800 ng/L increase the all-cause death rates [11]. It is worth noting that all mentioned risk factors of poor outcome were observed in this case study, which made the patient's prognosis even worse. The mismatch between LV wall thickness on ecoCG (1.3 cm) and autopsy (1.8 cm) observed in this patient is rather a pattern than an exclusion. According to the literature, the diagnostic efficiency of intravital identification of LV hypertrophy at postmortem measurement was low: the difference between autopsy and echoCG results for LV wall thickness was 3.3 to 5.2 mm, and for IVS — from 1.3 to 1.4 mm. This phenomenon can be caused by postmortem changes in the heart, which should be taken into consideration when diagnosing heart pathologies [12]. Unfortunately, despite numerous hospitalisations, neither healthcare professionals at the sanatorium, nor at the CDH, nor at the republican hospitals were able to suspect systemic amyloidosis, which, in our opinion, is a sign of their poor awareness. Early and prompt diagnosis of AC where amyloidosis is suspected is the top priority and a key to efficient therapy, since early therapy initiation can prevent further deposition of amyloid and progressive damage to target organs [1, 3]. Up to 2018, the only possible therapy of AC at ATTR was heart transplantation, the availability of which was significantly limited due to elderly age of patients and the need to transplant several organs (heart and liver). A breakthrough in the ATTR therapy was recently introduced and approved drug therapies, based on TTR gene inhibition (TTR protein synthesis inhibition), or TTR stabilisation (prevention of TTR tetramers dissociation to fibrils) [13].

In this case, the right moment was lost, and even despite the scientific breakthrough in the management of such patients with specific therapies [14], the patient was fated to die. Two days of hospitalisation after AC diagnosis was made did not make it possible to identify the type of amyloid while the patient was alive, and initiate specific therapy.

Conclusions

This clinical case emphasises the need for raising awareness of primary care providers about systemic amyloidosis in elderly patients, its diagnostic criteria and possible use of disease-specific therapy. Patients with AC need personalised diagnostic and therapeutic approach by a multidiscipline team of healthcare providers to develop a therapeutic strategy and access the potential role of available therapies to manage CF. Due to significant achievements in modern healthcare for the management of this category of patients, timely diagnosis will help in prompt therapeutic interventions to improve survival rates, physical functioning and/or quality of patients' life.

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

Soldatova O.V.: manuscript editing, drafting articles, approval of the final status of articles, interpretation clinical case data, search of references

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ХРОНИЧЕСКАЯ СЕРДЕЧНАЯ НЕДОСТАТОЧНОСТЬ С СОХРАНЕННОЙ ФРАКЦИЕЙ ВЫБРОСА У КОМОРБИДНОГО ПАЦИЕНТА: ВОПРОСЫ ВЕРИФИКАЦИИ «СЛОЖНОГО» ДИАГНОЗА

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Chronic Heart Failure with Preserved Ejection Fraction in A Comorbid Patient: Issues in Verification of A «Difficult» Diagnosis

Резюме

Хроническая сердечная недостаточность с сохраненной фракцией выброса (ХСНсФВ) — это сложный, гетерогенный, полиорганный системный синдром, который характеризуется значительной заболеваемостью и смертностью. В настоящее время он приобрел характер эпидемии XXI века. В клиническом наблюдении описана история пациентки пожилого возраста, страдающей ишемической болезнью сердца (ИБС), дислипидемией, стенозирующим атеросклерозом брахиоцефальных артерий на фоне артериальной гипертензии (АГ), ожирения, сахарного диабета (СД) 2 типа, осложненного диабетической ретинопатией, полинейропатией, нефропатией с развитием хронической болезни почек (ХБП) и ХСНсФВ. Состояние отягощалось наличием хронического пиелонефрита единственной почки после нефрэктомии справа по поводу абсцесса почки, бронхиальной астмы. Продемонстрированы ограничения в использовании современных шкал для определения предтестовой вероятности ХСНсФВ, низкий показатель натрийуретических пептидов (НУП). Коморбидность, плохо контролируемые АГ, СД, низкая приверженность к терапии привели к развитию острой сосудистой катастрофы, повторное нарушение мозгового кровообращения — к летальному исходу. Гистологически обнаружен выраженный периваскулярный и интерстициальный склероз в миокарде и эпикарде, который является основой диастолической дисфункции при ХСНсФВ.

Клинический пример отражает трудности диагностики ХСНсФВ, а также взаимное патогенетическое влияние сопутствующей патологии, что привело к неблагоприятному исходу при несоблюдении рекомендаций.

Ключевые слова: *хроническая сердечная недостаточность, сохраненная фракция выброса, фиброз миокарда, коморбидность*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

Источники финансирования

Авторы заявляют об отсутствии финансирования при проведении исследования

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Информированное согласие не требуется в силу невозможности идентифицировать пациента

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Abstract

Chronic heart failure with preserved ejection fraction (HFpEF) is a complex, heterogeneous, multi-organ systemic syndrome characterized by significant morbidity and mortality. Currently, it has acquired the character of an epidemic of the 21st century.

The clinical observation describes a typical story of an elderly patient suffering from coronary artery disease (CAD), dyslipidemia, atherosclerosis of the brachiocephalic arteries against the background of arterial hypertension (AH), obesity, type 2 diabetes mellitus (DM), complicated by diabetic retinopathy, polyneuropathy, nephropathy with development of chronic kidney disease (CKD) and HFpEF. The condition was aggravated by the presence of chronic pyelonephritis of a single kidney (right nephrectomy for renal abscess in 2013), bronchial asthma. The limitations of modern scales for determining the pre-test probability of HFpEF and low natriuretic peptide levels are demonstrated. Comorbidity, poorly controlled hypertension, diabetes, low adherence to therapy led to the development of acute vascular accident, then, repeated cerebrovascular accident — to a fatal outcome. Histologically, perivascular and interstitial sclerosis in the myocardium and epicardium was detected, which is the basis of diastolic dysfunction in HFpEF.

A clinical example reflects the difficulties of verification of HFpEF-diagnosis, as well as the mutual pathogenetic influence of concomitant pathology, which can lead to an unfavorable outcome if recommendations are not followed.

Key words: *chronic heart failure, preserved ejection fraction, myocardial fibrosis, comorbidity*

Conflict of interests

The authors declare no conflict of interests

Conformity with the principles of ethics

Informed consent is not required due to the impossibility of identifying the patient

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AH — arterial hypertension, BP — blood pressure, IHD — ischaemic heart disease, BMI — body mass index, LV — left ventricle, NUP — natriuretic peptides, DM — diabetes mellitus, LVEF — left ventricular ejection fraction, FC — functional class, CKD — chronic kidney disease, CCFwEF — chronic heart failure with preserved ejection fraction, echoCG — echocardiography

Introduction

A comorbidity (especially DM, AH, obesity and atrial fibrillation) is an underlying factor for CCFwEF. Nowadays, the number of patients with excessive body weight, impaired lipid and carbohydrate metabolism, renal dysfunction is constantly growing; these conditions trigger cellular and molecular changes in cardiac tissues: inflammation; fibrosis; impaired nitrogen oxide synthesis; sarcomere dysfunction; mitochondrial and metabolic diseases, which result in diastolic dysfunction, a key component in the development of CCFwEF [1]. Clinical phenotyping of CCFwEF is essential for selection of an individualised approach to the therapy. Patients with cardiac-renal-metabolic phenotype who have obesity, IHD, DM2, CKD, have poor prognosis and are at a high risk of all-cause death of hospital admission due to CCF [2]. Higher acute cardiovascular mortality rates in patients with abdominal obesity, dyslipidaemia, AH and hyperglycaemia are associated with endothelial dysfunction caused by insulin resistance, which is a universal vascular wall defect [3]. CCFwEF diagnosis in the real clinical practice is often challenging. Current clinical recommendations regulate diagnosis based on specific complaints, confimred with objective signs (or reaction to diuretics), markers of left ventricle diastolic dysfunction seen on echoCG, as well as higher natriuretic peptide levels. It is recommended to use H2FPEF and HFA-PEFF scales; and if there are minor dystolic dysfunction and other contradictory examination results, diastolic stress test should be used, which is not readily available in our country [4].

We are discussing a case study here, which reflects the characteristics and challenges with CCFwEF diagnosis in a cardiac-renal-metabolic patient.

Case Study

Here is the medical record of Patient S. (Figure 1), who was followed up in the outpatient clinic of City Clinical Hospital No. 11. In November 2023, the patient started complaining of shortness of breath and retrosternal pain after physical exercises, elevated blood pressure (BP) of up to 160/100 mm Hg, headache, general weakness, occasional ankle swelling.

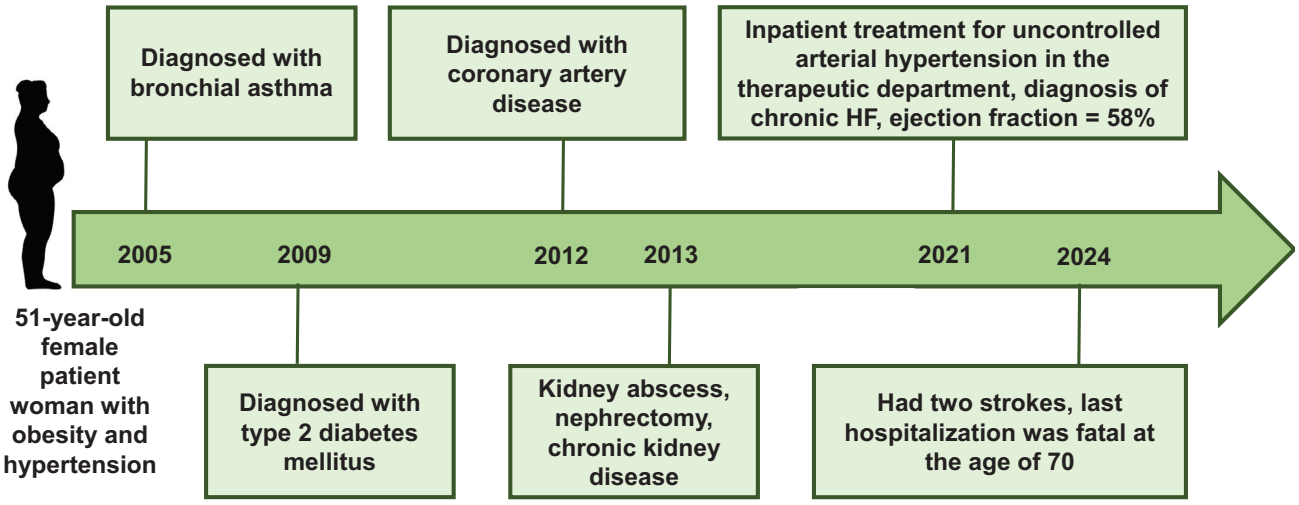

Figure 1. *Timeline of development of the cardiorenometabolic continuum and its outcomes in the observed patient*

According to her medical record, the patient had been suffering from hypertension since 1997; she did not take any regular antihypertensive drugs, usually when her BP was elevated. Then she was diagnosed with broncial asthma, which was treated with budesonide + formoterol (partial control of symptoms was achieved). Later she was diagnosed with DM2, her medications included various combinations of metformin, glibenclamide, gliclazide, vildagliptin. The patient was diagnosed with IHD, exertional angina, functional class (FC) II; she was taking acetylsalicylic acid, statins, nitroglycerine when needed, courses of trimetazidine, nicorandil. CKD developed as a result of BP, DM and prior right nephrectomy because of an abscess. In 2021, she was treated in an inpatient internal medicine ward of City Clinical Hospital No. 11, where CCFwEF was diagnosed for the first time (left ventricle ejection fraction (LVEF) = 58 %). The patient was not hospitalised any more, her condition worsened from November 2023. Life history: disabled person of group 2 from 2011; during the follow-up, she worked as a receptionist in a musical school (work in day-long shifts). Objective findings: height — 150 cm, weight — 85 kg, body mass index (BMI) — 37.7 kg/m^2. Waist circumference: 114 cm. Oxygen saturation is 98 %. The condition is satisfactory, and consciousness is clear. Vesicular breathing with individual dry rales in lower sections with forced expiration. Respiratory rate is 18/minute. Auscultation of the heart: muffled heart tones, regular rhythm; heart rate is 64 bpm; BP: 150/90 mm Hg. The abdomen is soft and non-tender on palpation. Ankle and feet pastosity. Bowel and bladder functions are within normal. Laboratory and instrumental test results: N-terminal pro B type natriuretic peptide (NT-proBNP) (31/10/2023) — 106.8 pg/mL; complete blood count (28/11/2023): elevated erythrocyte sedimentation rate (25 mm/h), otherwise unremarkable; urinalysis (27/11/2023): protein traces, albuminuria test strip testing: 20 mg/L. Blood biochemistry (27/11/2023): total cholesterol 7.2 mmol/L, high density lipoproteids 1.3 mmol/L, low density lipoproteids 4.9 mmol/L, plasma glucose 8.1 mmol/L, glicated haemoglobin 7.9 %, creatinine 112 μmol/L, glomerular filtration rate 43.2 mL/min/1.73m^2. Electrocardiography (27/11/2023): axis deviation to the left, sinus rhythm with heart rate of 70–77 bpm, signs of left ventricular (LV) hypertrophy, diffuse changes in the miocardium. Annual chest X-ray fluorography: no pathological shadows. Diagnosis: IHD, exertional angina, FC 2. Atherosclerosis of aorta and brachiocephalic arteries. Stage 3 hypertension, uncontrolled. LV hypertrophy. Degree 2 obesity. Dyslipidemia. Type 2 diabetes mellitus, diabetic retinopathy, polyneuropathy, nephropathy. Stage 3B CKD, stage A1 albuminuria. Risk grade 4 (very high). Stage 2A CCFwEF, FC 2 (LVEF 64 %). Chronic pyelonephritis of the remaining kidney (right nephrectomy because of an abscess, 2013). Mild persistent bronchial asthma (mixed genesis), controlled. Chronic cholecystitis, remission. Dorsopathy resulting from lumbar osteochondrosis, remission. The patient was consulted by a cardiologist, endocrinologist, lung specialist; commendations: losartan 100 mg/day; bisoprolol 2.5 mg/day; nitroglycerine 0.5 mg as required; torasemide 2.5 mg/day; atorvastatin 40 mg/day; acetylsalicylic acid 100 mg/day; metformin 2,000 mg/day; gliclazide 60 mg/day; vildagliptin 100 mg/day; budesonide + formoterol 400 + 12 μg/dose, 2 inhalations daily; ipratropium bromide + fenoterol 20 + 50 μg/dose, 2 inhalations as required. Patient's Morisky-Green (MMAS-8) compliance score is four points, i.e. poor compliance. After torasemide therapy, the patient noted improvement in swelling, shortness of breath after physical exercises, and discontinued the drug by herself one month later. She explained the inconsistencies in taking the medications and insufficient dosages by a large number of prescribed products, complicated dosage regimens, high cost of therapy, and irregularities in supply of subsidized

drugs. In March 2024, the patient underwent brachiocephalic artery duplex ultrasound scanning (11/03/2024): signs of atherosclerosis of brachiocephalic arteries, stenotic left internal carotid artery (up to 65 %) in its proximal section. Left vertebral artery hypoplasy. EchoCG (11/03/2024): LV end-diastolic diameter — 4.9 cm; LV end-sistolic diameter — 3.2 cm; LV end-diastolic volume — 110 mL; LV end-sistolic volume — 39 mL; LVEF — 64 %; interventricular septum thickness — 1.4 cm; diastolic LV posterior wass thickness — 0.9 cm; LV myocardial mass index — 116 g/m^2, relative LV wall thickness — 0.56; left atrium — 43*54*38 mm; indexed left atrium volume — 27.7 mL/m^2, pulmonary artery systolic pressure — 20 mm Hg; E/e' — 14. 6-minute walking test: 389 m, corresponding to FC 2. H2FPEF score: 5 points; HFA-PEFF score: 2 points. Intermediate probability of CCFwEF [4]. On 17/04/2024, the patient visited the emergency room at the outpatient clinic complaining of high blood pressure (160/100 mm Hg), headache, dizziness, shortness of breath during walking. Her antihypertensive therapy was corrected: amlodipine 2.5 mg/day and moxonidine 0.2 mg/day were added, a sick note was initiated because of uncontrolled AH. When on 22/04/2024 her blood pressure rose to 190/100 mm Hg, the patient started experiencing unsteady gait, blackouts, weakness in her lower extremities; she did not call for ambulance, as her next GP and endocrinologist appointments were due on 23/04/2024. Consultation by GP dated 23/04/2024: BP 160/100 mm Hg, talks with difficulties, marked weakness in her limbs, unsteady gait. Electrocardiography findings: no signs of acute coronary pathology. The neurologist urgently referred the patient to the neural vascular ward at City Clinical Hospital No. 11 with suspected acute cerebrovascular event, where the patient was treated from 23/04/2024 to 13/05/2024 for ischemic stroke in the vertebrobasilar system with dysarthria, atherothrombotic subtype, with secondary brainstem syndrome. EchCG (23/04/2024): LVEF — 60 %, E/e' — 14, left atrium volume index — 24 mL/m^2, left ventricle myocardium mass index — 110 g/мм^2, pulmonary artery systolic pressure — 20 mm Hg. The patient was discharged for the follow-up by neurologist and GP; recommendations: lisinopril 20 mg/day, amlodipine 5 mg/day, atorvastatin 40 mg/day, ezetemibe 10 mg/day, acetylsalicylic acid 100 mg/day, gliclazide 90 mg/day, sitagliptin 100 mg/day. Home visit (14/05/2024): stays is bed, double incontinence, quadriparesis, dysarthria, requires assistance. Since on 18/05/2024 the patient's condition and speech function deteriorated, her shortness of breath at rest worsened, the patient was admitted to the neural vascular ward at City Clinical Hospital No. 11 and connected to an invasive mechanical ventilation apparatus, where she died on 19/05/2024. A postmortem examination revealed ischaemic brainstem stroke; myocardial hypertrophy (heart weight: 398 g, left ventricle wall thickness: 1.6 cm, interventricular

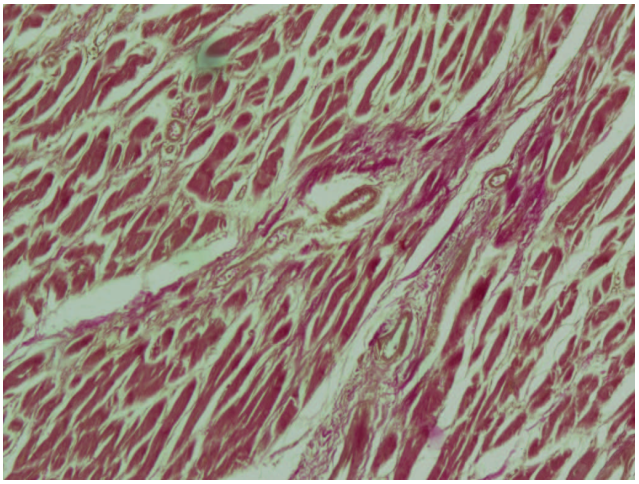

Figure 2. *Fragment of the myocardium.*
Note. Van Gieson's staining. The connective tissue in the perivascular and interstitial zone turned bright pink. Edema of the interstitium and hypertrophy of cardiomyocytes

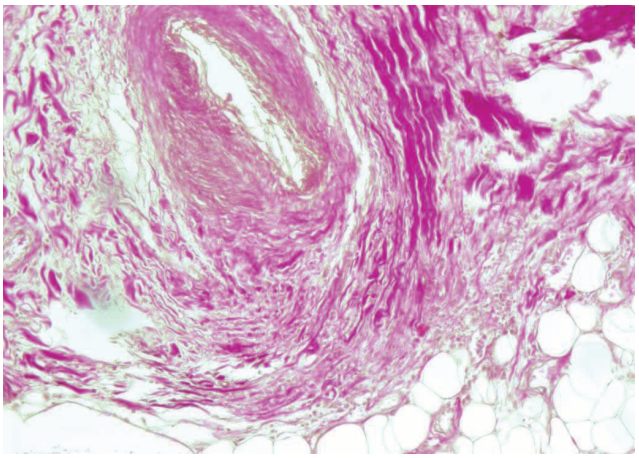

Figure 3. *Fragment of the epicardium*
Note. Van Gieson's staining. The connective tissue in the perivascular zone turned bright pink (severe sclerosis of the vessel walls)

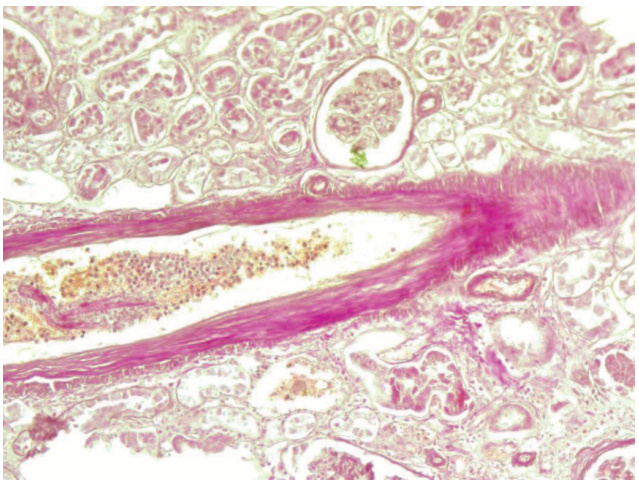

Figure 4. *A kidney fragment*
Note. Van Gieson's staining. The connective tissue in the perivascular zone and the connective tissue in the stroma of the organ. Necrobiosis of the tubular epithelium

septum: 1.6 cm); enlarged cardiac cavities; macrofocal cardiosclerosis of the lower wall of the left ventricle (old grey 2.2 cm scar); obliterating atherosclerosis of coronary arteries (grade 3, stage 3, stenosis 56 %); chronic general congestion of internal organs: nutmeg liver, congestive splenomegaly, swelling of subcutaneous tissue of lower limbs; chronic pyelonephritis attack. Heart tissue histology showed marked interstitial and perivascular fibrosis (Figure 2, Figure 3).

Discussion

Patients with CCFwEF have comorbidities; every second patient has over five non-cardiac comorbidities; the process is triggered by a combination of risk factors and associated conditions, including age, female sex, hypodynamia, obesity, atrial fibrillation, IHD, DM, dyslipidemia, AH, metabolic syndrome, CKD, anaemia, chronic obstructive pulmonary disease, and sleep apnea. There are no specific diseases, which could be a clear cause of CCFwEF, since this condition is inflammatory or metabolic [5]. There are no specific symptoms or signs of CCFwEF; they are similar to those in patients with cardiac failure: shortness of breath, poor tolerance of physical activities, fatigue, chest discomfort [6]. The patient had signs and symptoms of CCF, diastolic dysfunction seen on echo CG, which, even in the absence of elevated NT-proBNP confirms CCFwEF, despite insufficient HFA–PEFF and H2FPEF score [4]. In the real-time clinical practice, diagnostic algorithms are often not used due to NUP testing unavailability, poor availability of modern ultrasound units at outpatient clinics, and impossibility for the functional diagnostics specialists to examine the diastole in detail. Thorough echoCG examination is fundamental for the diagnosis of diastolic dysfunction and CCFwEF verification. Myocardial fibrosis caused by inflammatory and metabolic disorders makes cardiac cavities more rigid, resulting in higher LV filling pressure and left atrium pressure. A non-invasive marker of this process is E/e' — a ratio between the force, the left atrium has to overcome to fill LV with blood during diastole, and the rate of LV relaxation. However, during echoCG only 20.6 % of functional diagnosis specialists measure Simpson's LVEF; 13.5 % still use the Teicholz method; 62.6 % use both methods, and 3.2 % don't measure LVEF at all. Indexed left atrium volume is routinely measured by 56.8 % of medical professionals, E/e' is measured by 51.6 %; echoCG speckle-tracking and diastolic stress test are rare. Under these circumstances, symptomatic comorbid patients with obesity, DM, atrial fibrillation, CKD are in the grey area for the assessment of the probability of CCFwEF using modern scales [7, 8]. Clinical measurements of NUP values shifted the paradigm in the management of CCF patients; however, NT-proBNP is not an ideal biomarker of CCFwEF. The value ruling out this condition is below 125 pg/mL; and patient previously treated for CCF can have lower values [4]. There are causes of elevated NUP levels: elderly age, CKD, acute coronary syndrome, pulmonary hypertension, pulmonary embolism, transient elevation during initiation of valsartan + sacubitril, use of cardiotoxic medicines, atrial fibrillation and other arrhythmias, sepsis, thyrotoxicosis, valvular diseases. Literature references actively discuss the NUP deficit syndrome; there are numerous factors affecting their reduction in CCF patients: obesity, pulmonary oedema, chronic cardiac compression, cardiac tamponade, gene *NPPB* polymorphism, elevated androgen levels in female patients, hypercorticoidism, insulin resistance, continuous valsartan + sacubitril therapy, elevated androgen levels, minor problems with myocardium structure and function [9, 10]. The exact feedback mechanism between obesity and NT-proBNP levels is still unknown. It is assumed that obesity increases NUP clearance in adipocytes, which can be programmed on genetical level and reduces their release. It is thought that lower NUP levels are recorded in patients with CCFwEF and obesity because of increased mechanical load for the pericardium (similar to chronic cardiac compression) [6, 11, 12]. Therefore, the current recommendation is to reduce baseline NT-proBNP values by 25 % for BMI of 30 to 35 kg/m^2; by 30 % for BMI of 35 to 40 kg/m^2, and by 40 % for BMI over 40 kg/m^2 [13]. According to this recommendation, in stage 2 obesity, the lower NT-proBNP level for the patient was 87.5 pg/mL. Also, she had factors increasing NT-proBNP levels: elderly age and CKD. Cardiac-renal-metabolic CCFwEF is widely presented in numerous studies; these are patient with obesity, DM, CKD; they can account for 1/3 of the CCFwEF population [2]. Developing metabolic disorders and inflammatory process play an important role in CCFwEF pathogenesis, and the relationship between these two biological processes is termed appropriately — metaflammation, or inflammatory-fibrous paradigm [14, 15]. Obesity, which is associated with constant low-intensity systemic inflammation, triggers a cascade of cardiac-renal-metabolic syndrome, promotes myocardium infiltration with monocytes, which release inflammatory cytokines activating fibroblasts and later causing myocardial fibrosis, underlying CCFwEF development [16, 17]. By triggering insulin resistance, obesity promotes DM development, which is diagnosed in every third patient with CCF, irrespective of impaired glucose tolerance, fasting glycemia and pre-diabetes. Even more marked microvascular inflammation in DM patients results in diffuse atherosclerosis-independent myocardial fibrosis. In uncontrolled glycemia, endothelial dysfunction worsens, vascular wall is affected by atherosclerosis, which can later result in acute circulation failure [18]. DM leads to significant reduction in NT-proBNP levels, which is a result of its higher glycosilation, which is promoted by higher blood glucose levels and insulin resistance in obese individuals [11]. DM, obesity and AH underlie CKD.

Table 1. *Management considerations for patients with a cardiorenal-metabolic profile and comorbid conditions*

Issue	Approach
Weight management and glycemic control	Low-calorie diet, increased physical activity (aerobic exercises), and consideration of GLP-1 receptor agonists. If eGFR >30 ml/min/1.73m^2, SGLT2i and metformin are the primary options [4].
Achieving target blood pressure	Avoid short-acting antihypertensive drugs. In patients with asthma, CCB and ARB are preferable, as they are less likely than ACEi to induce cough [20, 21].
Preserving kidney function	Initiate treatment with low doses and titrate up carefully while monitoring eGFR and electrolytes. Avoid any medications without prior consultation with a physician [4, 20].
Lipid disorders and atherosclerosis	Statins should be used to achieve LDL targets (<1.4 mmol/L or a 50 % reduction from baseline). If statins are insufficient, ezetimibe should be added [4, 21].
Bronchial asthma management	Regular use of maintenance therapy is essential. Avoid short-acting β-agonists due to their adverse cardiovascular effects, systemic glucocorticoids (which negatively impact glucose and lipid metabolism), and β-blockers unless absolutely necessary, using only at low doses [20, 21].
Patient education and preventive care	Participation in diabetes education programs and heart failure management clinics. Referral for stage 3 medical rehabilitation and routine medical follow-ups. Vaccination against influenza and pneumococcal infections [4].

Abbreviations: GLP-1 — Glucagon-like peptide-1 receptor agonists, eGFR — estimated Glomerular Filtration Rate, SGLT2i — Sodium-glucose co-transporter 2 inhibitors, CCB — Calcium channel blockers, ARB — Angiotensin receptor blockers, ACEi — Angiotensin-converting enzyme inhibitors, LDL — Low-density lipoproteins

General pathological mechanisms, general risk factors or systemic impairments affect the heart and kidneys, causing their simultaneous dysfunction. Every second CCF patient has CKD. Organ hypoperfusion resulting from inadequate heart functioning activates the renin-angiotensin-aldosterone and sympathic nervous system, oxidative stress, necrobiotic and fibrotic changes in the kidney, resulting in the development and active progression of CKD. The patient had chronic type 2 cardiorenal syndrome: impaired cardiac function caused by AH, IHD, DM, CCF, leading to progressive kidney injury; nephrectomy because of an abscess was an important factor for CKD development, which affected the renal function [19, 20].

When observing this interconnected pathological cascade, it becomes clear that monitoring blood glucose levels, BP and lipid levels in a comorbid patient is very important, and the patients should be aware not only of their diseases, but also understand that the prescribed therapy improves prognosis and prolongs their life. A disease-modifying therapy in CCFwEF patients should include sodium glucose linked co-transporter-2, renin-angiotensin-aldosterone system inhibitors, mineralocorticoid receptor antagonists [4]. Since BP was not controlled adequately, losartan replacement with candesartan, or valsartan + sacubitril should have been discussed, aldosterone antagonists should have been added [4, 21]. Failure to reach target levels of low-density lipoprotein in the patient from the very high risk group with obliterating atherosclerosis of brachiocephalic arteries requires addition of ezetemibe to the statin. Since the patient had bronchial asthma, her heart rate should have been controlled with ivabradine or dihydropyridine calcium antagonists (amlodipine/ felodipine) instead of beta blockers [4]. A combination of the mentioned medications would have provided optimal heart and kidney protection.

Conclusion

This case study demonstrates challenges with CCFwEF verification in a comorbid patient with cardiac-renal-metabolic phenotype in outpatient settings due to low NUP levels and lack of ultrasound criteria for reliable diagnosis, which restricts the use of scales and algorithms for CCFwEF verification in real-time clinical practice. Timely diagnosis and drug correction of a comorbidity, prescription of disease-modifying therapy and better compliance are the factors, which can improve prognosis.

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Пономарева О.В.: разработка концепции, написание текста рукописи, сбор данных и обработка материала, взаимодействие с редакцией в процессе подготовки публикации и печати

Смирнова Е.А.: научное руководство, разработка концепции, критический пересмотр статьи на предмет важного интеллектуального содержания, доработка текста статьи, итоговые выводы.

Шукис К.А.: проведение аутопсии, подготовка и описание гистологических микропрепаратов, предоставление иллюстративного материала, итоговые выводы.

Author Contribution:

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

Ponomareva O.V.: concept development, manuscript writing, data collection and material processing, interaction with the editors during the preparation of the publication and printing.

Smirnova E.A.: scientific supervision, concept development, critical revision of the article for important intellectual content, revision of the article text, final conclusions.

Shukis K.A.: autopsy, preparation and description of histological micropreparations, provision of illustrative material, final conclusions.

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