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# STUDY OF THE ROLE OF OXIDATIVE STRESS, ANTIOXIDANT PROTECTION AND IMMUNE INFLAMMATION MARKERS IN THE PATHOGENESIS OF CHRONIC HEART FAILURE BY THE MIDDLE RANGE EJECTION FRACTION IN ELDERLY PATIENTS DEPENDING ON GENDER

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**ABSTRACT:**Of particular interest is the study of the mechanisms of development of chronic heart failure (CHF), especially with middle range ejection fraction in elderly patients.65 patients were examined (40 men, 25 women), age 66.7 ± 4.3 years, with chronic heart failure middle range ejection fraction (HFmrEF), NYHA class II, control group (CG) - 30 age-comparable individuals without signs of heart failure. The concentration in the blood of oxidized low-density lipoproteins (OxLDL) in patients with HFmrEF (112.3 (96.3; 123.6) ng/ml) is 98% higher than CG; women are 19% higher than men. The value of general oxidizing ability of the blood in the study group is 2.71 (2.38; 2.90) μmol/L, which is 51% higher than CG; in women it is 14% higher. In the HFmrEF group, the values of general antioxidant activity of blood are 40.7 (35.2; 50.7) μmol/L, which is 29%, lower than CG, in women 24% higher than men. The content of superoxide dismutase in patients with HFmrEF (0.54 (0.43; 0.71) ng/ml), which is 44% lower than CG, in women 25% higher. The level of interleukin-1β (interleukine) in HFmrEF patients (2.84 (2.35; 3.42) pg/ml) is 135% higher than CG, in women it is higher by 31%. The content of interleukin-6 in the blood of patients with HFmrEF (6.18 (5.38; 6.78) pg/ml), which is 2.27 times higher than CG, and interleukin-8 in patients with HFmrEF (22.6 (20, 3; 24.4) pg/ml) 6.38 times higher than CG. No gender differences were found. The level of tumor necrosis factor-α in the HFmrEF group is 3.49 times higher (14.02 (11.34; 15.37) pg/ml) than KG, in women it is 26% higher. The level of interleukin-4 in the HFmrEF group in women is 24% higher than men. In elderly patients with HFmrE, a state of oxidative stress, a decrease in antioxidant defense, an increase in immune inflammation, especially pronounced in women, were revealed.

**KEYWORDS:** elderly patients, chronic heart failure middle range ejection fraction, oxidative stress, antioxidant protection, immune inflammation, gender

## I. INTRODUCTION

Cardiovascular diseases are the most important cause of morbidity and mortality among older people worldwide. The frequency of cardiovascular diseases increases after 65 years in men and 75 years in women.

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Elderly patients have physiological changes caused by age, a large number of concomitant diseases, atherosclerotic diseases of the cardiovascular system.

The prevalence of chronic heart failure (HF) in various regions of the Russian Federation is 7–10% [1]. Over the past decade (from 1998 to 2014), the average age of patients with heart failure has increased from 64.0 ± 11.9 years to 69.9 ± 12.2 years [2].

Currently, of particular interest is the study of the mechanisms of development of heart failure with an intermediate ejection fraction, especially in elderly patients. In old age, changes in the cardiovascular system are associated with diastolic dysfunction of the left ventricle (LV), an afterload increase on myocardium, and rigidity of the large artery. At the cellular level, they include oxidative stress, chronic inflammation, changes in the intercellular matrix [3, 4].

At present, the role of the rennin-angiotensin-aldosterone system, sympatho -adrenal, endothelin, and cytokine systems in the pathogenesis of HF has been proven [5, 6]. The increase in the blood of patients with heart failure with proinflammatory cytokines confirms the presence of immune inflammation.

The pathogenetic mechanisms detected by atherosclerosis - dyslipidemia, oxidative stress - are accompanied by activation of proinflammatory blood cells. Blood concentrations of interleukin-1β (IL) and tumor necrosis factor alpha (TNF-α) are used as markers of CHF severity. A number of studies have demonstrated an increase in the level of pro-inflammatory cytokines in the blood, regardless of the etiology of heart failure [7].

The results of several studies have shown a direct relationship between elevated plasma levels of inflammatory cytokines and changes in the functional class HF by NYHA and the ejection fraction of the LV (LV). It was found that increased cytokine production is associated with cardiomyocyte hypertrophy, apoptosis and the development of fibrosis, which, in turn, is the basis for the progression of heart failure [7].

In 2016, the European recommendations separately highlighted a new group of heart failure - heart failure with mid-range ejection fraction (HFmrEF) from 40 to 49% [8], and therefore work on the features of the pathogenetic mechanism and progression heart failure are single, and studies conducted in patients with heart failure, depending on age, are absent.

Objective: to assess the role of oxidative stress, antioxidant defense, and markers of immune inflammation in the pathogenesis of HFrEF in elderly patients, depending on gender.

## II. MATERIAL AND METHODS

An open randomized study was conducted, involving 65 patients with HFmrEF NYHA class II (40 men, 25 women), the average age was 66.7 ± 4.3 years. The control group (CG) consisted of 30 individuals, of the same age and gender, who, according to studies, lacked objective signs of heart failure.

The determination of total oxidative status (TOS) was carried out by an enzymatic test using reagents from Labor Diagnostika Nord GmbH KG (Germany). Oxidized low density lipoproteins (OxLDL) were measured by a similar method with reagents from Biomedica (Germany). The general antioxidant activity of blood serum (GAA) was measured using an iMark plate reader, (USA) reagents from CanAg Diagnostics AB (Sweden). Superoxide dismutase (SOD) (Superoxide dismutase) (Cu / Zn form) was determined using the Superoxide Dismutase Assay Kit (USA). Determination of the level of interleukins (IL) IL-1β, -4, -6, -8, TNF-α (TNF-α) was carried out using a set of reagents Merck Millipore, (Germany). All studies used the method of enzyme-linked immunosorbent assay.

Quantitative data are presented as median (Me) and Q25%; Q75%. The differences were considered significant at $p < 0.05$.

## III. RESULTS AND DISCUSSION

The state of lipid peroxidation and the antioxidant system of the body plays an important role in the pathogenesis of both coronary heart disease and heart failure (HF). The following parameters were analyzed: the concentration in the blood of oxidized low-density lipoproteins (OxLDL), the total oxidative status of blood serum (TOS), the general antioxidant activity (GAA) of the blood, and the activity of superoxide dismutase (SOD).

Analysis of the results of the study showed that in patients with CG the blood concentration of the most aterogenic OxLDL was 98% lower and amounted to 56.8 (49.7; 66.2) ng/ml compared with patients with

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HFmrEF in which this indicator was 112.3 (96.3; 123.6) ng/ml (p <0.001). In the HFmrEF group (Fig. 1), OxLDL in men was lower than in women (p <0.01).

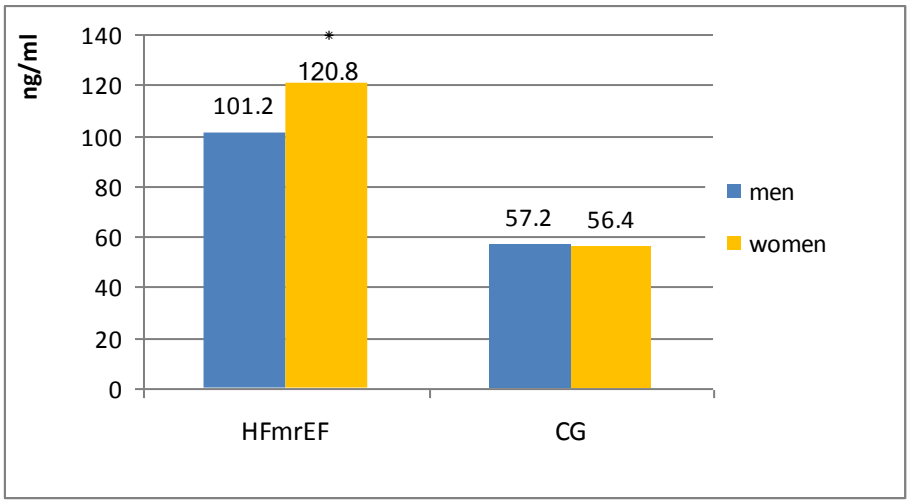


**Fig. 1 OxLDL level depending on sex with HFmrEF and CG (* - differences between men and women are significant at p < 0.05)**

When analyzing OOS, it was revealed that in the CG the indicator is 1.79 (1.68; 1.89) mmol/L., In patients with HFmrEF the values were - 2.71 (2.38; 2.90) mmol/L., which 51% higher (p <0.001).

As one of the most important components of the antioxidant system of blood, we considered the SOD enzyme (SOD), the content of which in the examined patients with HFmrEF is 0.54 (0.43; 0.71) ng/ml, which is 44% less than in KG - 0 91 (0.78; 0.96) ng/ml, (p <0.001).

A comparative analysis of the state of the lipid peroxidation system and the antioxidant blood system in men and women with HFmrEF is presented in Table 1.

**Table 1 :Indicators of oxidative stress and antioxidant protection of blood in patients with HFmrEF and in CG, takinggender into account**

| | | OxLDL, ng/ml | TOS, μmol/l | GAA, μmol/L | SOD, ng/ml |
|---|---|---|---|---|---|
| HFmrEF, n= 65 ppl | Men | 101.2 (96.3;107.2)*# | 2.45 (2.38;2.69)*# | 38.1 (34.6;42.8)*# | 0.52 (0.48;0.59) *# |
| | Women | 120.8 (114.3;123.6)* | 2.79 (2.62;2.90)* | 47.1 (41.3;51.1)* | 0.65 (0.58;0.69)* |
| CG n= 30 ppl | Men | 57.2 (49.7;63.1) | 1.80 (1.74;1.87) | 57.8 (56.4;63.4) | 0.92 (0.84;0.96) |
| | Women | 56.4 (53.4;66.2) | 1.75 (1.68;1.89) | 56.9 (52.3;61.5) | 0.88 (0.78;0.94) |

Note: * - differences with CG are significant at p <0.001; # - intragroup differences between men and women are significant at p <0.05

In the control group, the level of OxLDL did not differ depending on gender (p > 0.05). In the group of patients with HFmrEF, the concentration of OxLDL in the blood in women was higher - 120.8 (114.3; 123.6) ng/ml than in men 101.2 (96.3; 107.2) ng/ml (p < 0.001).

The serum TOS in men and women of the CG did not have significant differences (p > 0.05). In the group of patients with HFmrEF, the values in women were 14% higher than in men (p < 0.01).

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The total antioxidant activity of blood in men and women of the CG did not have significant differences ($p > 0.05$). In the group of patients with HFmrEF, the blood GAA value in women was 24% higher than in men ($p < 0.01$).

Blood SOD level in men (0.92 (0.84; 0.96) ng/ml) and women (0.88 (0.78; 0.94) ng/ml) CG did not have significant differences ($p > 0.05$). In the group of HFmrEF, the level of SOD in women is 25% higher than in men ($p < 0.01$).

Thus, in patients with HFmrEF, a state of oxidative stress was established. This is due, on the one hand, to an increase in blood TOS and OxLDL concentration, and, on the other hand, to a weakening of the antioxidant defense mechanisms of the body, as indicated by a decrease in serum GAA and a decrease in SOD. In women with HFmrEF, in comparison with men, a more pronounced activity of both oxidative processes and antioxidant defense mechanisms was determined.

Atherosclerotic lesion of the coronary arteries, which is detected in patients with HFmrEF, is associated with chronic subclinical inflammation. We studied IL-1β, -6, -8, TNF-α as pro-inflammatory cytokines, and IL-4, anti-inflammatory.

In CG patients, the level of IL-1β in the blood was 1.21 (1.12; 1.32) pg/ml, in HFmrEF 135% higher - 2.84 (2.35; 3.42) pg/ml, ($p < 0.001$).

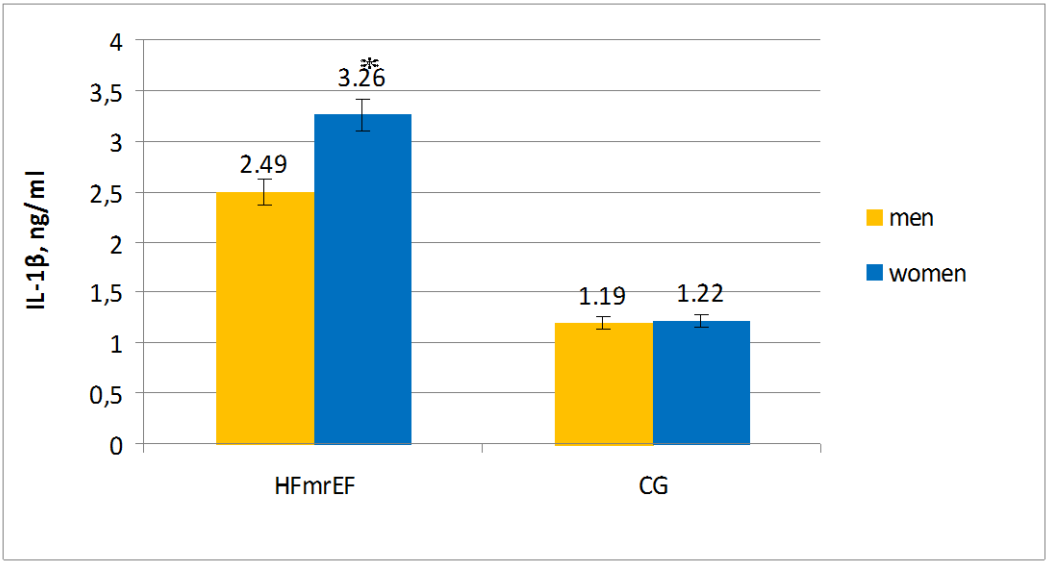


**Figure 2 shows the levels of IL-1β in the blood of patients with HFmrEF and CG, depending on gender.**

Fig. 2 The level of blood IL-1β depending on gender with HFmrEF and CG (* - differences between men and women are significant at $p < 0.05$)

In the CG, this indicator did not have significant differences depending on gender ($p > 0.05$). In patients with HFmrEF, the level of IL-1β was 31% higher in women - 3.26 (2.88; 3.42) pg/ml than in men - 2.49 (2.35; 2.69) pg/ml ($p < 0.001$).

Blood IL-6 level was 2.27 times lower in patients with CG - 2.72 (2.38; 3.21) pg/ml than in patients with HFmrEF 6.18 (5.38; 6.78) pg/ml ($p < 0.001$). Table 2 shows the results of the analysis of gender differences in the level of IL-6 blood, indicating the absence of statistically significant differences in gender in relation to the studied cytokine. In the CG, the level of blood IL-8 was - 3.54 (3.31; 3.94) pg/ml, in patients with HFmrEF the content was 6.38 times higher - 22.6 (20.3; 24.4) pg/ml ($p < 0.001$). After analyzing the data on the content of IL-8 in blood in patients with HFmrEF and in individuals with CG, depending on gender, we did not reveal differences in this indicator (Table 2). It was established that in the CH the level of TNF-α blood was 4.02 (3.58; 4.87) pg/ml, in patients with HFmrEF - 14.02 (11.34; 15.37) pg/ml, which is 3, 49 times higher than CG.

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**Table 2: The level of IL-6, IL-8 blood in patients with HFmrEF and in persons of the control group**

| Groups | | The content of IL-6, pg/ml | The content of IL-8, pg/ml | The content of IL-4, pg/ml |
|---|---|---|---|---|
| HFmrEF | men | 6.12 (5.38; 6.71)* | 22.2 (20.3; 24.4)* | 4.14 (3.91; 4.78)* |
| | women | 6.26 (5.61; 6.78)* | 22.8 (20.4; 24.2)* | 5.11 (4.70; 5.39) *# |
| CG | men | 2.74 (2.44; 3.21) | 3.67 (3.38; 3.94) | 4.70 (4.12; 5.24) |
| | women | 2.68 (2.38; 3.19) | 3.49 (3.31; 3.68) | 5.49 (5.17; 6.16) # |

Note: * - differences with the control group are significant at $p < 0.01$; # - intragroup differences between men and women are significant at $p < 0.05$

Features of the production of TNF-α were also studied in men and women with HFmrEF, and in CG (Fig. 3). In the CG, there were no gender differences in the level of TNF-α ($p > 0.05$). In patients with HFmrEF, its blood concentration was significantly higher in women - 15.21 (14.85; 15.37) pg/ml than in men 12.03 (11.34; 13.87) pg/ml in ($p < 0.01$). As a cytokine with anti-inflammatory activity, we evaluated the concentration of IL-4 in the blood in individuals of the compared groups (Table 2).

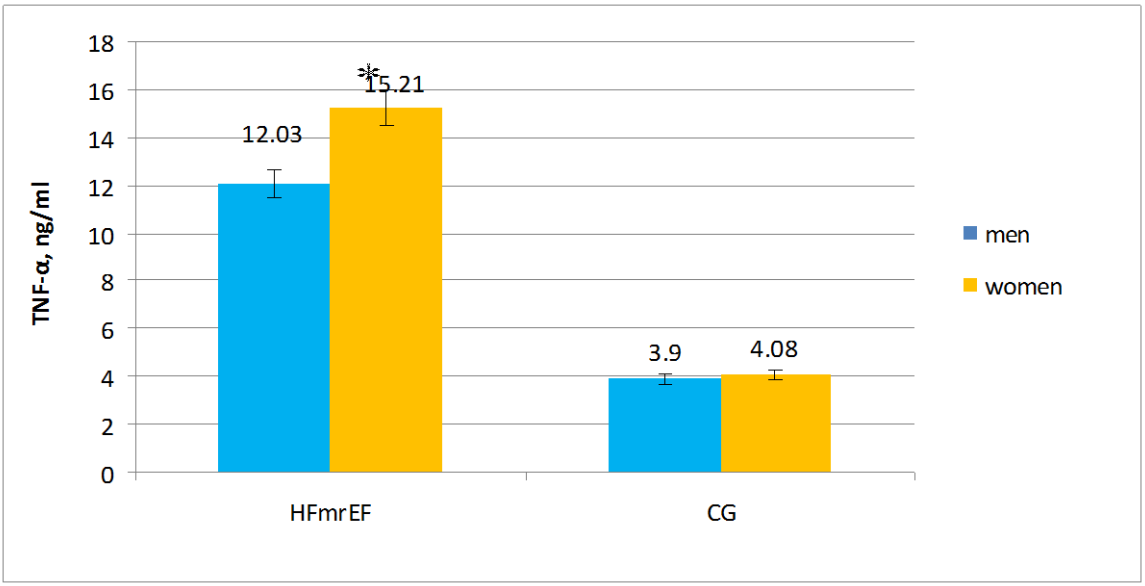


**Fig. 3. The level of TNF-α in the blood in men and women with HFmrEF and CG (* - differences between men and women are significant at $p < 0.05$)**

The decrease in the level of IL-4 in patients with HFmrEF compared with CG was 11% ($p > 0.05$).Data on IL-4 in the examined individuals depending on gender are shown in Figure 4.

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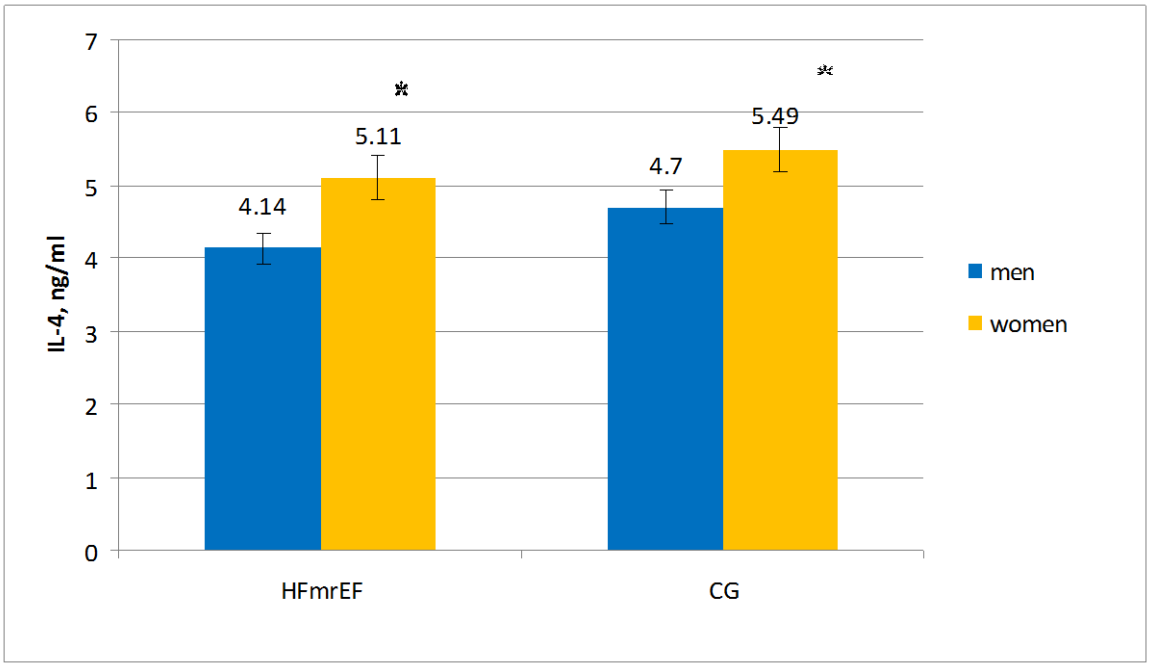


**Fig. 4. The level of IL-4 blood depending on gender with HFmrEF and in the control group (* - differences between men and women are significant at p <0.05)**

In all groups, the level of IL-4 blood was significantly higher in women compared with the value of this indicator in men (Fig. 4). In women with IL, the concentration of IL-4 was 5.49 (5.17; 6.16) pg/ml, and in men 4.70 (4.18; 5.24) pg/ml ($p < 0.01$). In patients with HFmrEF, having generally lower values of IL-4, its level in womn - 5.11 (4.70; 5.39) pg/ml was 24% higher than in men - 4.14 (3, 91; 4.78) pg/ml ($p < 0.01$).

Thus, the results of the analysis of the study of blood cytokine status indicate that in patients suffering from ischemic etiology HFmrEF, an increase in the activity of the pro-inflammatory system of interleukins is observed against the background of a decrease in the activity of anti-inflammatory mechanisms.

The study allows us to highlight the features of the cytokine profile of blood associated with the gender of patients. It was found that women with HFmrEF have higher levels of IL-1β and TNF-α, which is associated with increased pro-inflammatory activity. With respect to IL-6, -8 no gender differences were found. IL-4 production was significantly higher in women with both HFmrEF and CG.

Among the mechanisms of influence on the course of heart failure, coronary atherosclerosis, coronary heart disease (coronary heart disease), an increase in the activity of pro-inflammatory cytokines that potentiate chronic subclinical inflammation, oxidative stress, which accelerated platelet aggregation by 3 times, is currently considered.As the results of our study showed, the blood TOS value in patients with HFmrEF exceeded that in CG individuals by 51%, in women by 14% higher than in men.

An important consequence of the formation of free radicals is the oxidation of LDL. It is known that the modification of LDL sharply increases their atherogenicity [9]. It was found that in patients with HFmrEF, the level of oxLDL was 198% higher than in the CG, which contributes to an increase in the atherogenic potential of blood in the examined category of patients.

As components that counteract oxidative stress in patients with HFmrEF, we evaluated blood GAA and the level of SOD. It was found that the decrease in the activity of the antioxidant system of blood in the group of HFmrEF by 71% compared with KG. The blood SOD content in the HFmrEF group is 44% lower than in the CG. The increase in blood TOS naturally led to an increase in the concentration of oxLDL ($R = 0.54$), which are also an atherogenic factor. Simultaneously with an increase in the activity of oxidative processes in patients with HFmrEF, a decrease in blood GAA level ($R = -0.54$) and SOD concentration ($R = -0.54$) was noted.

The pathophysiological features of HFmrEF of ischemic etiology are the repeated processes of ischemia, in which free radicals are not utilized enough by the components of antioxidant protection due to a decrease in SOD activity. In the early stages of CHF development, a compensatory increase in the activity of SOD is noted,

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which is replaced by its inhibition at later stages and with age. In the study, we studied the state of the cytokine status of blood as an indicator of chronic inflammation in patients with HFmrEF.

An analysis of the data found that patients with HFmrEF had a higher blood content of IL-1β by 135%, IL-6 2.27 times, IL-8 6.38 times higher than in CG. The content of TNF-α in the blood of the HFmrEF group in comparison with CG also increased - by 3.49 times. A comparative assessment of the level of IL-4 showed that in patients with HFmrEF its concentration in the blood was 11% lower than in patients with CG.

Hyperproduction of IL-1β enhances the development of atherosclerosis, the synthesis of other cytokines involved in inflammation, prostaglandins and collagen.

Our data indicating an increase in the level of IL-6 are consistent with information obtained by other authors on the role of IL-6 as a factor in the interleukocyte interaction involved in the pathogenesis of atherosclerosis. Chronic inflammation in CHF is accompanied by the development of endothelial dysfunction, as evidenced by a decrease in the production of nitric oxide with increasing concentration in the blood of IL-6 [10]. Of great clinical importance for understanding the participation of cytokines in the pathogenesis of atherosclerosis is the ability of IL-6 to stimulate the synthesis of atherogenic particles such as very low-density lipoproteins and triglycerides by hepatocytes [11].

In patients with HFmrEF, as shown by our studies, there is an increased production of IL-8, which, as you know, increases under the influence of IL-1β, TNF-α and other factors. Among the functions of IL-8, mediation of the inflammatory response in cardiovascular diseases is noted [12].

The decrease in the production of anti-inflammatory IL-4 detected by us in patients with HFmrEF of ischemic etiology in comparison with CG is of great interest. It is known that IL-4 is a differentiation factor for T and B lymphocytes [13], but its role as a cytokine with anti-inflammatory activity in the pathogenesis of cardiovascular diseases has not been fully studied. Single reports indicate an increased risk of developing coronary heart disease in individuals with IL-4 gene polymorphism, a relationship between IL-4 deficiency and heart remodeling in hypertension.

The next area of analysis of the obtained data was the study of the gender characteristics of the studied parameters in elderly patients with HFmrEF and patients in the control group. We have identified some gender differences in the studied group. It was revealed that in women with HFmrEF, the level of oxLDL exceeds this indicator in men by 19%, the level of blood TOS in women also exceeds the value in men by 14%.

The fact that we obtained regarding the activity of the antioxidant system is of particular interest: in women with HFmrEF it was higher than in men. So, the content of SOD in the blood in women was 25% higher than the concentration of the enzyme in men, the level of blood GAA was 24% higher than the value of this indicator in men.

In connection with the identification of systemic subclinical inflammation, we analyzed the cytokine status of the blood, depending on the gender of the patients. Significant gender differences in blood levels of pro-inflammatory cytokines - IL-6, IL-8 were not detected.

Regarding IL-1β, it was found that its level did not differ in patients of different sexes in the CG, howeer, in women with HFmrEF it was higher by 135% than in men of this group.

A similar situation was obtained when studying the level of TNF-α: in the absence of gender differences in the concentration of this interleukin in the blood of people with CG, in the group of patients with HFmrEF the level of TNF-α was significantly (26%) higher in women than in men.

A study of the blood content of a cytokine with anti-inflammatory properties of IL-4 showed that in the control group and in the groups of patients with HFmrEF its levels were higher in women compared with men by an average of 24%.

## IV. CONCLUSION

In elderly patients with chronic heart failure with middle range ejection fraction revealed a state of oxidative stress, a decrease in antioxidant protection, an increase in the activity of chronic immune inflammation, especially manifested in women.

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