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Inflammatory Biomarkers in Patients With Type 2 Diabetes Mellitus and Heart Failure With Preserved Ejection Faction

Aim	To verify the relationship between gene polymorphisms of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) with inflammation markers and codependent metabolic variables in patients with type 2 diabetes mellitus and chronic heart failure (CHF).
Material and methods	This study included 154 patients (mean age, 69.1±3.2 years). The control group consisted of 47 patients with metabolic syndrome (MS) without CHF; the 2nd group included 56 patients with CHF with preserved ejection fraction (CHFpEF); and the 3rd group consisted of 51 patients with CHF with reduced ejection fraction (CHFrEF). The rs1800629 polymorphism of the TNF-α gene (TNF-α: G308A) was studied in real time by the polymerase chain reaction (PCR) method and the rs1800795 polymorphism of the IL-6 gene (IL-6: 174 G>C) was studied by PCR with the electrophoretic detection. The frequencies of polymorphic alleles were compared with the clinical blood test results, plasma concentrations of C-reactive protein (CRP), TNF-α, leptin, and fibrinogen. Differences between the groups were determined using the F test. Relationships between individual studied parameters were identified using the regression analysis.
Results	In most patients, the occurrence of gene polymorphisms was eident as increased plasma concentrations of biomarkers. An association was found between the TNF-α gene polymorphism (G308A) and an increase in plasma TNF-α and between the IL-6 gene polymorphism (174 C>G) and an increase in plasma CRP. In the CHFpEF group, the rs1800629 gene polymorphism was observed in 55% of patients, among whom 93% had increased TNF-α. The rs1800795 gene polymorphism was observed in 82% of CHFpEF patients, among whom 21% had increased CRP. In the CHFrEF group, the G308A transition in the TNF-α gene was observed in 53% of patients; an increase in the respective cytokine was noted in 67% of patients; the IL-6 gene polymorphism 174 C>G was found in 78%, however, only 14% of patients with this polymorphism had also increased CRP. In the control group, the TNF-α G308A gene polymorphism was found in 30% of patients, while an increase in free TNF-α was associated with this polymorphism in 50% of patients; the IL-6174 C>G gene polymorphism was detected in 78%, while no increase in the CRP level was observed in this group. This demonstrates a high probability of the TNF-α G308A gene polymorphism occurrence in patients with CHF.
Conclusion	Inflammatory markers are important predictors of CHF. The most significant predictor was the TNF-α G308A gene polymorphism, which was observed in more than 50% of patients, the majority of whom had an increase in plasma TNF-α.

Keywords	Chronic heart failure; left ventricular ejection fraction; inflammatory cytokines; gene polymorphism; rs1800629, rs1800795
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Introduction

In recent years, there has been a growing interest among researchers in the role of pro-inflammatory cytokines in the pathogenesis of cardiovascular diseases (CVD), notably chronic heart failure (CHF), the treatment of which represents a significant challenge in modern clinical cardiology [1]. It has been observed that genetic predisposition plays a role in the activation of alternative molecular mechanisms involved in the pro-inflammatory cascades that cause pathological myocardial remodeling and contribute to the progression of CVD [2]. The activation of the immune system and the presence

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of systemic inflammation serve as early predictors of the development of CVD as well as the progression and decompensation of CHF. A negative correlation between the expression of the wild-type tumor necrosis factor alpha (TNF-α) gene and left ventricular ejection fraction (LVEF) has been previously identified. The expression of wild-type alleles of the TNF-α and interleukin-6 (IL-6) genes, as well as the levels of these cytokines, have been demonstrated to be elevated in patients with CHF. A positive correlation has been identified between the concentrations of TNF-α and IL-6 and the New York Heart Association (NYHA) classes of CHF. The concentrations of TNF-α and IL-6 are elevated in patients with CHF class III–IV in comparison to patients with CHF class I–II and healthy subjects [3].

The cytokines TNF-α and IL-6 act as a trigger for the development of systemic oxidative stress, which in turn leads to the onset of coronary heart disease (CHD) and CHF. [4]. It is frequently observed that patients with CHF exhibit elevated plasma concentrations of pro-inflammatory cytokines [5]. The findings of recent meta-analyses indicate that certain polymorphisms of the TNF-α and IL-6 genes are both independent predictors of CHF and factors that influence its phenotype [2, 6, 7].

A comparison of 43 patients with CHF of ischemic origin and 140 healthy individuals has revealed a significantly higher frequency of the TNF-α-238A/A genotype in the patient group. However, no differences have been observed in the frequencies of IL-6 alleles and genotypes. It should be noted that the authors have not controlled for LVEF [5]. In a separate study, the recessive genotypes of two single nucleotide polymorphisms of the C-reactive protein (CRP) gene (rs1800947 and rs11265263) have been linked to an elevated risk of mortality and elevated CRP levels. However, no association has been observed between the IL-6 gene polymorphism and mortality [8].

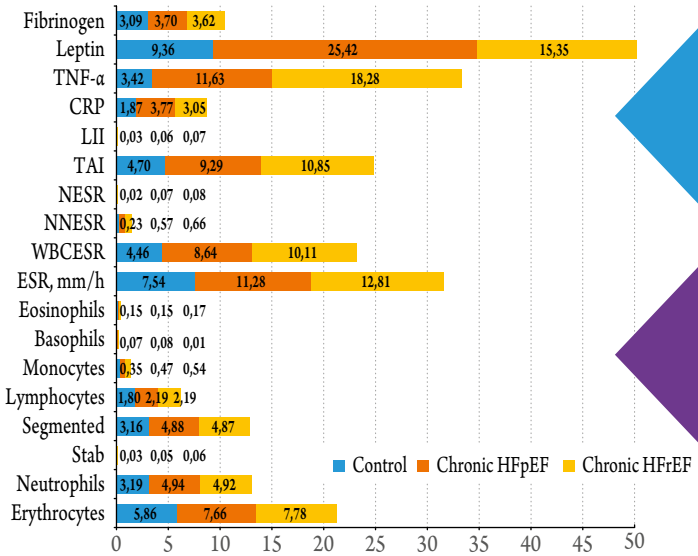
The reviewed papers demonstrate a definitive involvement of pro-inflammatory cytokines in the development of CHF. However, the authors do not consider the discrepancies in the CHF phenotypes, which may contribute to the contradictory results obser-

Central illustration. Inflammatory Biomarkers in Patients With Type 2 Diabetes Mellitus and Heart Failure With Preserved Ejection Faction

PCR analysis of gene polymorphism frequencies: TNF-α: 308G>Ars1800629 Interleukin-6: 174G>C rs1800795

Control group – 47 patients with metabolic syndrome but without chronic heart failure (CHF)
Group 2 – 56 patients with CHF with preserved ejection fraction (HFpEF)
Group 3 – 51 patients with CHF with reduced ejection fraction (HFrEF)

Mean age 69.1 ± 3.2 years



The population statistical method was employed to determine (illegible) polymorphic alleles, clinical (illegible) plasma concentrations of C-reactive protein (illegible) leptin and fibrinogen, calculated indicators (illegible)

Group differences were determined by F-test (illegible) polymorphic gene frequencies and parameters (illegible) calculated indicators of inflammation were used to reveal (illegible) regression analysis.

Signs of chronic inflammation were seen in both study groups, but not in the control group. The inflammation (illegible) more pronounced in the HFpEF group than in the HFrEF group. In the HFpEF group, the regression analysis found (missing part of the text) IL-6 polymorphisms: 174 C>G and TNF-α G308A, but not in the control group and the HFrEF group. The most significant (illegible) was TNF-α G308A, which was observed in 53% of patients with elevated TNF-α levels in the blood (illegible).

The HFpEF and HFrEF groups are different clinical phenotypes.

ved. Accordingly, we have undertaken an evaluation of the impact of TNF-α and IL-6 gene polymorphisms on the development of inflammation and, potentially, subsequent CHF in patients with type 2 diabetes mellitus (DM).

Objective

The objective of this study is to verify the association between the TNF-α and IL-6 gene polymorphisms and inflammatory markers and co-dependent metabolic parameters in patients with type 2 diabetes mellitus and congestive heart failure.

Material and Methods

The study cohort comprised 154 patients (mean age 69.1±3.2 years) from the clinic of internal medicine at the Kirov Military Medical Academy, Kirov Interdistrict Clinical Hospital, and City Diabetes Center No. 2.

Inclusion criteria: presence of type 2 diabetes (at least five-year history of the disease) and/or metabolic syndrome (MS) and CHF with preserved (at least 50%) LVEF (HFpEF) or reduced (less than 40%) LVEF (HFrEF); signing a voluntary informed consent form for participation in the study.

Exclusion criteria: refusal to participate in the study, existing valve pathology, cardiomyopathy, atrial fibrillation/atrial flutter, storage diseases, cancer, infectious and autoimmune diseases, respiratory system diseases, obstructive sleep apnea syndrome, anemia, or chronic kidney disease. The study did not include patients with moderately reduced LVEF (40–49%). Additionally, patients presenting with acute conditions and diseases necessitating surgical intervention were excluded from the study.

The study design involved the administration of laboratory tests, clinical examinations, and molecular genetic analysis.

To identify individuals with chronic HFpEF, the level of the N-terminal pro-brain natriuretic peptide (NT-proBNP) was determined in all patients. Patients with NT-proBNP levels exceeding 125 pg/mL were included in the study. To determine LVEF and the presence of diastolic dysfunction, all patients underwent echocardiography and diastolic stress test. The final analysis included three groups: a control group comprising 47 patients with MS without CHF, a HFpEF group comprising 56 patients, and a HFrEF group comprising 51 patients. The patients in each group exhibited grade 1–2 obesity, arterial hypertension (AH), and, in the case of the HFrEF group only, CHD. Patients were treated with background therapy for pre-existing diseases, including CHF, in accordance with the current clinical guidelines. The patient cohort was comparable in terms of sex, age, body mass index, and disease duration (AH, type 2 DM/MS). The anamnestic, physical, and biological data were collected in accordance with the GCP guidelines. The study protocol was subjected to a review and subsequently approved by the local ethics committee of the Kirov Military Medical Academy (minutes #271, dated November 22, 2022).

Genomic DNA was extracted and purified from whole blood using DNA-Technology kits (Russia). The quality of the isolated DNA was evaluated using a Nanodrop 2000C spectrophotometer (Thermoscientific, USA). The samples were subsequently stored at a temperature of –20 °C until they were subjected to analysis. The rs1800629 gene polymorphism (TNF-α: G308A) was investigated through polymerase chain reaction (PCR) with real-time fluorescent product detection, using the Litech reagents (Russia) and DT-Prime 5 amplifier (DNA-Technology, Russia). A single nucleotide substitution in rs1800795 (IL-6: 174 G>C) was identified through a PCR with electrophoretic detection. For the PCR, we utilized Litech reagents and a four channel thermostat TP4 PCR-01-TERTSIK (DNA-Technology, Russia). The amplification products were separated in a 3% agarose gel in TAE buffer by horizontal electrophoresis. A solution of ethidium bromide at a concentration of 1% (equivalent to 10 μL per 100 mL of melted gel) was added as a fluorescent dye. The PowerPac HC (Bio-Rad, USA) was employed as the power source for the electrophoresis process. The results were obtained using a ChemiDoc MP Imaging System (Bio-Rad, USA) and Image Lab Software (Bio-Rad, USA).

The polymorphic allele frequencies were compared with complete blood counts, including erythrocyte sedimentation rate (ESR), plasma concentrations of CRP, TNF-α, leptin, and fibrinogen. Complete blood counts and ESR were employed to calculate indices that characterize inflammatory processes.

$$\text{WBCESR} = \frac{\text{WBC count} \times \text{ESR}}{10},$$

normal – up to 4.

$$\text{NESR} = \frac{(\text{stab neutrophils} + \text{segmented neutrophils}) \times \text{ESR}}{100},$$

normal – up to 3.8.

$$\text{NNESR} = \frac{\text{non-segmented neutrophils} \times \text{ESR}}{10},$$

normal – up to 2.3.

$$\text{TAI} = \Lambda\text{WBCESR} + \text{NESR} + \text{NNESR},$$

normal – up to 10.3.

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$$LII = \frac{\text{WBC count} \times (\text{stab} + \text{juvenile} + \text{myelocytes})}{\text{segmented} + \text{lymphocytes}},$$

normal – 1.0–1.2 units

where: WBCESR, white blood cells and ESR; NESR, neutrophils and ESR; NNESR, non-segmented neutrophils and ESR; TAI, total activity index; LII, leukocyte inflammation index.

The statistical processing of the obtained data was conducted using the StatSoft Statistica 10 program, employing descriptive statistical methods. The data are presented as median values with 95% confidence intervals (CI). To ascertain group differences, the Mann-Whitney U-test and the Wilcoxon test were employed. In order to evaluate the consistency of the data, the coefficient of variation was calculated. This is defined as the ratio of the mean square deviation to the mean, expressed as a percentage.

$$V_\sigma = \sigma / x \cdot 100\%,$$

where: Vσ, coefficient of variation; σ, mean square deviation; X, mean.

The degree of data dispersion is considered insignificant if the coefficient of variation is less than 10%. If the value is between 10% and 20%, the dispersion is considered moderate. If the value is greater than 20% and less than or equal to 33%, the dispersion is considered significant. A coefficient of variation value of less than or equal to 33% indicates a homogeneous population, while a value exceeding 33% suggests a heterogeneous population. Consequently, if the coefficient of variation exceeds 33%, it can be concluded that the distribution within the sample is not normal.

A regression analysis was employed to determine the correlation between the individual parameters. For this purpose, the paired text variables denoting allelic variants

Table 1. Complete blood counts of the patients examined

Parameter	Control group			Chronic HFpEF group			Chronic HFrEF group			p		
	Me	95 %CI	Q	Me	95 %CI	Q	Me	95 %CI	Q	Comparison of the HFrEF group and the control group	Comparison of the HFrEF group and the control group	Comparison of the HFpEF group and the HFrEF group
Leukocytes, ×109/L	5.7	1.07–1.61	21.96	7.70	1.26–1.83	19.46	7.66	1.47–2.18	22.56	0.2713	0.9362	0.3681
Neutrophils, n	3.4	0.93–1.40	35.16	4.62	1.29–1.88	31.00	4.59	1.31–1.93	31.68	0.0001	0.0001	0.2713
Stab cells, %	0.03	0.01–0.01	59.00	0.06	0.04–0.06	76.32	0.05	0.02–0.03	43.13	0.0414	0.0324	0.5287
Segmented neutrophils, %	3.37	0.92–1.39	35.12	4.58	1.27–1.84	30.85	4.54	1.29–1.91	31.65	0.0238	0.0044	0.3472
Lymphocytes, %	1.70	0.42–0.64	28.13	2.04	0.73–1.06	39.62	2.04	0.59–0.87	32.07	0.1770	0.2420	0.5687
Monocytes, %	0.30	0.11–0.17	39.00	0.48	0.14–0.20	34.83	0.48	0.17–0.26	38.13	0.0394	0.0271	0.6818
Basophils, %	0.03	0.10–0.16	164.00	0.06	0.35–0.65	358.29	0.01	0.04–0.07	328.80	0.2150	0.2757	0.5552
Eosinophils, %	0.16	0.08–0.12	59.04	0.15	0.09–0.14	80.00	0.14	0.11–0.17	93.40	0.4009	0.4965	0.9203
ESR, mm/h	6.5	4.16–6.26	66.27	10.0	4.88–7.09	51.25	11.00	4.46–6.6	41.55	0.0001	0.0000	0.0767
WBCESR	3.46	2.53–3.81	68.25	8.03	3.73–5.41	51.04	9.08	4.66–6.89	54.95	0.0108	0.0045	0.6455
NESR	0.18	0.14–0.21	70.99	0.51	0.29–0.42	60.07	0.61	0.37–0.55	66.69	0.1164	0.0193	0.4839
NNESR	0.02	0.01–0.02	87.45	0.08	0.06–0.09	96.14	0.06	0.04–0.06	67.66	0.0203	0.0232	0.4777
TAI	3.72	2.64–3.98	67.54	8.59	4.03–5.86	51.42	9.69	5.02–7.43	55.27	0.0010	0.0027	0.6455
LII	0.04	0.01–0.02	60.46	0.06	0.05–0.08	85.18	0.05	0.02–0.03	43.61	0.0285	0.0340	0.4065
CRP, mg/L	1.90	0.77–1.15	49.11	3.70	1.35–1.96	42.35	2.80	1.55–2.30	60.70	0.0076	0.0143	0.9045
TNF-α, pg/mL	3.15	2.13–3.48	77.24	10.08	3.97–5.81	40.51	20.40	5.31–8.28	35.40	0.0000	0.0000	0.0001
Leptin, ng/mL	10.20	5.48–9.01	72.77	24.60	11.27–16.74	52.98	12.59	6.60–10.12	52.03	0.0131	0.0394	0.5353
Fibrinogen, g/L	3.04	0.52–0.82	20.53	3.72	0.67–1.00	21.75	3.59	0.53–0.80	17.59	0.0005	0.0045	0.3125

HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; CI, confidence interval; CV, coefficient of variation; IL-6, interleukin-6; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; TNF-α, tumor necrosis factor alpha.

of polymorphisms were subjected to coding, whereby homozygous wild allele was designated as 1, heterozygous polymorphic allele as 2, and homozygous polymorphic allele as 3. Given that the codes represent a segment of a natural series that is a continuous, linear, monotonically increasing, and infinite progression, a linear regression was deemed an appropriate fundamental model. The observed differences were deemed to be statistically significant at the $p < 0.05$ level.

Results

Leukocyte counts in patients with chronic HFpEF and chronic HFrEF were increased 1.31-fold and 1.33-fold, respectively, in comparison with the control group. Nevertheless, these values remained within the physiological normal range (Table 1). Although the data exhibits minimal variability, as indicated by the coefficients of variation (CV), the observed differences with the control group are statistically insignificant. Given that the neutrophil count in the HFpEF and HFrEF groups is 1.55 times that observed in the control group, the mean number of individual forms of neutrophils in these groups is statistically significantly elevated relative to the control group, specifically 2 times higher in HFpEF patients. The number of lymphocytes in the HFpEF and HFrEF groups is 1.21 and 1.22 times higher, respectively, than in the control group. However, there are no statistically significant differences, which is evidently attributable to the considerable variability of the data. The monocyte count was found to be statistically significantly higher (1.54 times) in patients with HFrEF compared to the control group. There was considerable variation in basophil counts (Table 1), which is why the observed differences between the groups are statistically insignificant. It is noteworthy that eosinophil counts are slightly reduced in the HFpEF and HFrEF groups relative to the control group. ESR was increased by a factor of 1.5 and 1.7, respectively, in comparison to the control group, and the resulting differences were statistically significant.

The elevation of the calculated indices of active inflammation by a factor of two or more (in comparison to the control group) is a distinctive feature of the HFpEF and HFrEF groups (Table 2). Notably, NESR does not differ between the control and HFpEF groups due to a high degree of variation, despite an increase by a factor of 2.5. This reaction is accompanied by a statistically significant increase in CRP and TNF-α concentrations in both the HFpEF and HFrEF groups. The concentration of TNF-α in patients with HFrEF was 5.34 times higher than that observed in the control group and 1.57 times higher than that observed in the HFpEF group. Concurrently, the blood concentration of leptin in patients with HFpEF was 2.72 times higher than that observed in the control group, representing a statistically significant increase. In contrast, an insignificant increase was observed in this indicator in patients with HFrEF. The fibrinogen concentrations observed in both study groups were higher than that observed in the control group. However, the differences were not statistically significant.

Table 2. Correlations between gene polymorphisms and clinical and laboratory data of patients in the control group

Dependent predictor	Independent predictor	R2	F	p
Leptin	Leukocytes	0.8131	14.501	0.0037
	IL-6 gene polymorphism 174 C>G			0.0013
	TNF-α			0.0040
	Lymphocytes			0.0085
	LII			0.0124
	NESR			0.0065
Basophils	Eosinophils	0.1871	8.285	0.0067

R^2 is the coefficient of determination, the proportion of the variance of the dependent variable. IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; LII, leukocyte inflammation index; NESR, neutrophils + erythrocyte sedimentation rate.

Although the overall mean values are within the reference range, the concentration of CRP exceeds the upper threshold (reference interval 0.0–5.0 mg/L) in 16% of patients with HFpEF and in 14% of patients with HFrEF. In contrast, the control group exhibited values within the reference interval. Elevated fibrinogen concentrations (reference interval: 2.0–3.9 g/L) were observed in 36% of patients with chronic HFpEF, 32% of patients in the HFrEF group, and only 6% of patients without CHF. TNF-α concentrations (normal <8.1 pg/mL) are elevated in 85% of patients with HFpEF, 96% of patients with HFrEF, and only 35% of patients without CHF. The differences are statistically significant when compared to both the control group and between the HFpEF and HFrEF groups. Leptin levels are elevated in 95% of patients with HFpEF, 86% of patients with HFrEF, and 51% of patients without HFrEF (normally with levels ranging from 3.7 to 11.1 ng/mL in women and from 2 to 5.6 ng/mL in men).

In regression analysis, the dependent predictor, designated as «Group,» is found to be correlated with two independent predictors: «TNF-α» ($R2 = 0.562$; $\beta = 0.72$; $p = 0.0000$) and «Fibrinogen.» ($\beta = 0.15$; $p = 0.0217$). The strength of the correlation is strong ($F = 68.637$).

In the control group (Table 2), the dependent predictor «Leptin» is correlated with independent predictors «Leukocytes» ($\beta = –0.38$), IL-6 polymorphism: 174 C>G ($\beta = 0.42$), «TNF-α» ($\beta = 0.38$), «Lymphocytes» ($\beta = 0.39$), «LII» ($\beta = 0.37$), «NESR» ($\beta = 0.35$). An inverse correlation was identified between the predictors «Basophils» and «Eosinophils» ($\beta = –0.43$), which confirms the negative values of the regression coefficients. This indicates that an increase in the number of basophils is associated with a decrease in the number of eosinophils, and vice versa.

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Table 3. Correlations between gene polymorphisms and clinical and laboratory data of patients in the HFpEF group

Dependent predictor	Independent predictor	R2	F	P
Sex	Leptin	0.5219	26.193	0.0000
	IL-6 gene polymorphism: 174 C>G			0.0406
Leptin	Sex	0.9948	57.628	0.0044
	ESR			0.0008
	Monocytes			0.0117
	Leukocytes			0.0017
	IL-6 gene polymorphism: 174 C>G			0.0180
	TNF-α			0.0045
	Basophils			0.0046
	CRP			0.0045
	TNF-α gene polymorphism: G308A			0.0065
	Lymphocytes			0.0221
Basophils	CRP	0.4180	14.362	0.0011
Eosinophils	Leptin	0.7907	11.330	0.0067
	Basophils			0.0017
	TNF-α			0.0007
	Lymphocytes			0.0085
C-reactive protein	Basophils	0.9014	20.579	0.0007
	Leukocytes			0.0025
	ESR			0.0130
	Leptin			0.0455
TNF-α	Lymphocytes	0.9161	17.468	0.0001
	Eosinophils			0.0005
	Basophils			0.0021
	Leptin			0.0029
	Sex			0.0314

R2 is the coefficient of determination, the proportion of the variance of the dependent variable. HFpEF, heart failure with preserved ejection fraction; IL-6, interleukin-6; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; TNF-α, tumor necrosis factor alpha.

In the HFpEF group (Table 3) dependent predictor «Sex» is associated with leptin concentrations (β = 0.71) and frequency of IL-6 gene polymorphism: 174 C>G (β = –0.27). It is of particular significance that within this group, the dependent predictor «Leptin» correlates with significantly more independent predictors than in the control group, including both polymorphisms studied: sex (β = 2.11), ESR (β = 0.38), monocytes (β = –1.32), leukocytes (β = –0.47), IL-6: 174 C>G (β = 0.47), TNF-α (β = 0.67), basophils (β = –1.40), CRP (β = 1.76), TNF-α G308A (β = 0.38), lymphocytes (β = –0.29). In consideration of the magnitude of factor F, this correlation is more pronounced than that observed in the control group.

It was therefore possible to establish a relationship between the clinical and laboratory data and the frequencies of polymorphic genes only in the group of patients with HFpEF. It is noteworthy that the regression coefficients β between leptin concentrations and the number of monocytes, basophils and lymphocytes were negative. It may be the case that in patients with HFpEF, leptin concentrations represent the most significant factor in the activation and maintenance of inflammatory processes.

In the HFrEF group, the correlated predictors were «Basophils» and «CRP» (β = 0.65). The eosinophil count as a dependent predictor exhibit association with leptin (β = 0.46), basophils (β = 0.55), TNF-α (β = –0.72), and lymphocytes (β = 0.49). CRP is correlated with basophils (β = 0.61), leukocytes (β = 0.44), ESR (β = 0.37), and leptin (β = –0.27). TNF-α concentrations are associated with lymphocytes (β = 0.83), eosinophils (β = –0.95), basophils (β = 0.63), leptin (β = 0.80), and sex (β = –0.42). The correlation between the frequencies of TNF-α gene polymorphism has been established: G308A as a dependent predictor with the leukocyte count (F = 5.9122; R2 = 0.1057; β = –0.33; p = 0.0187).

It is important to note that a sufficient number of patients exhibited both the polymorphism and its manifestation in the form of an increase in biomarker concentration. Thus, it has been established that there is a correlation between the TNF-α gene polymorphism: G308A and the elevated TNF-α levels and between the IL-6 gene polymorphism: 174 C>G with an increase in CRP concentration. In the HFpEF group, the TNF-α gene polymorphism: G308A was found in 55% of patients, 93% of which exhibited an increase in the TNF-α levels. In the same group, the IL-6 gene polymorphism: 174 C>G was observed in 82% of patients, elevated levels of CRP in 21% of patients, primarily those with the G/G genotype.

In the HFrEF group, transitions of G308A in the TNF-α gene were observed in 53% of subjects, with elevated TNF-α levels reported in 67% of patients. Additionally, the IL-6 gene polymorphism: 174 C>G, was identified in 78% of subjects, while only 14% of patients with this polymorphism exhibited an increase in CRP levels. In the control group, the TNF-6 gene polymorphism: G308A was identified in 30% of patients, and elevated TNF-α associated with the polymorphism was reported in 50% of patients; the IL-6 gene polymorphism: 174 C>G was found in 78%; no elevation in CRP levels was observed in this group. These findings indicate a high likelihood of TNF-α gene polymorphism: G308A in patients with CHF.

Therefore, patients with HFpEF demonstrated the greatest number of correlations between the parameters examined in the regression analysis, which is indicative of the presence of a chronic inflammatory process.

Discussion

Leptin was the primary predictor associated with the phenotype of the patients examined in the control and HFpEF groups. The regression equations included independent predictors: leukocyte and lymphocyte counts, the IL-6 gene

polymorphism: 174 C>G, TNF-α levels (Tables 2 and 3). The contribution of TNF-α is thus contingent upon the activation of specific types of receptors with an affinity for it (TNF-αRs), namely TNF-αR1 (CD120α, p55) and TNF-αR2 (CD120β, p75). It has been demonstrated that the cardiodestructive effect of the mediator is due to the stimulation of TNF-αR1 (present on the surface of cardiomyocytes, fibroblasts, and endotheliocytes), while ligation of TNF-αR2 has been shown to induce cardioprotection [1]. It is crucial that the topography of the receptors is heterogeneous, and, with elevated cytokine levels, there is a further redistribution towards the first subtype (TNF-αR1), exacerbating potentially existing morphofunctional changes in the myocardium [9]. This is particularly evident in CHF when, with elevated TNF-α levels, the processes of atherosclerotic origin are exacerbated [4, 6] and the manifestations of decompensated CHF increase [1]. Direct damage to cardiomyocytes is caused by inducers of cell apoptosis formed during ligation of TNF-αR1, namely pyroptosomes (Complex IIa or IIb) and necrosomes (Complex IIc), which cause inflammation and necroptosis [10]. The continuous local inflammation results in pathological hypertrophy of the myocardial tissue, which is followed by dilatation of the heart chambers [9]. Concurrently, the resulting imbalance in calcium metabolism accelerates the decline in the contractility of the ventricular syncytium.

J.M. Barbosa-Ferreira et al. (2013) [11] systematically reviewed the data on the ability of leptin to modulate metabolism, respiratory control, and inflammation, all of which are factors associated with CVD. Previously, this was confirmed through direct observation of patients over a period of eight years. The results demonstrated a positive correlation between leptin concentration and the incidence of CHF and CVD [12]. The leptin/adiponectin index is a predictor of the efficacy of treatment for CHF or the probability of an unfavorable prognosis, which may ultimately result in a fatal outcome [13].

Despite the existence of compelling evidence indicating the involvement of leptin in the development of CHF, primarily gathered between 2001 and 2012, there has been a notable decline in the interest of researchers in investigating the potential role of this biomarker in the pathogenesis of CHF, which is substantiated by requests in the PubMed database. Our analysis demonstrates that in patients with type 2 DM and HFpEF, an increasing number of predictors of chronic systemic inflammation are involved in the pathogenesis, with leptin playing a predominant role. Furthermore, there is a correlation with sex. The latter, apparently has not been the subject of detailed study. However, it is known that in overweight men with CHF, compared to healthy individuals, the concentration of leptin determines the total content of minerals in the bones [14]. This suggests that leptin plays a pivotal role in predicting inflammation in patients with CHF and type 2 DM.

One of the final effects of inflammation at the cellular level is a qualitative and quantitative change in the leukocyte count. Our findings revealed a positive correlation between the number of eosinophils and leptin, TNF-α, and the number of basophils and lymphocytes. It was previously hypothesized that an investigation into the correlation between eosinophils, mast cells, and CHF would facilitate the identification of straightforward prognostic markers for CHF [15]. Thus, our findings can be seen as a logical extension of the cited research. It has been demonstrated that eosinophils play a role in the remodeling of the epithelium and subepithelial layers of smooth muscles during chronic inflammation. Additionally, they are involved in the formation of endomyocardial fibrosis and CHF [16]. In instances where polymorphisms are identified, the transcription process is enhanced due to alterations to the nucleotide sequence of promoter regions. Consequently, the implementation of polymorphisms is reduced to an increase in the concentration of relevant mediators that exacerbate the course of CHF either directly through cardiotoxicity or indirectly through the promotion of hemodynamic and metabolic disorders.

The IL-6 gene polymorphism, which is frequently linked to the stimulation of the production of acute phase inflammatory proteins, particularly CRP, was identified as an independent predictor in both the control and HFpEF groups. The central activating effect of IL-6 on the hypothalamic-pituitary-adrenal axis not only potentiates endocrinological disorders, particularly those affecting lipid and carbohydrate metabolism, but also myocardial «aging» (CHF) in the context of prolonged sympathetic hyperstimulation [17].

Additionally, metabolic disorders may result from the interference of IL-6 with leptin and insulin. This can lead to the excessive production of a mediator in obese patients with type 2 DM, which markedly elevates the risk of developing cardiovascular disease. S.D. Anker et al. (2004) [1] highlight the dualistic nature of the local (cardiac) action of IL-6, namely its capacity to initiate the hypertrophy of cardiomyocytes, subsequently leading to myocardial dysfunction and atrophy, and to inhibit their apoptosis [18]. The underlying cause may be attributed to the nature of the bioavailable fraction of TNF-α, in response to the release of which IL-6 is produced. Additionally, potential modifications in the gene encoding TNF-α may also be a contributing factor. Nevertheless, thus far there are no studies that either substantiate or disprove this hypothesis. It is postulated that genotypes 174 GG/GC are a predisposing factor for the development of CVD [19], particularly the GG genotype, which is associated with elevated levels of CRP, a marker for CHF.

A regression analysis of data obtained from patients in the HFrEF group revealed a positive association between the TNF-α gene polymorphism: G308A with leukocyte count. The result is explained from the perspective of knowledge regarding the regulatory function of autacoids. However, it does not permit the identification of the mechanisms underlying the development

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of this variant of the CHF phenotype in patients with type 2 DM. It can be concluded, however, that the formation of the HFrEF phenotype is generally less dependent on inflammatory processes.

Further research and application of modern biomarkers in clinical practice will enhance our comprehension of the underlying mechanisms of the studied diseases and facilitate more accurate risk stratification. Nevertheless, this study is subject to a number of limitations, including sample heterogeneity, a short follow-up period, and the number of patients. Consequently, further studies are required to assess the level of inflammatory markers both initially and during prospective follow-up. Repeated measurements can be employed to monitor the progression of the disease and, by extension, to evaluate the efficacy of the selected treatment strategies.

Conclusion

In both study groups, laboratory evidence of a chronic inflammatory process was observed in comparison with the control group. Nevertheless, in the group of patients with chronic heart failure and preserved left ventricular ejection fraction, the regression analysis revealed a significantly greater number of correlations between the examined laboratory parameters and interleukin-6 gene polymorphisms: 174 C>G and TNF-α: G308A compared to the control group and patients with chronic heart failure with reduced left ventricular ejection fraction. The inflammatory process in the group of patients with a preserved left ventricular ejection fraction is more pronounced than in those with a reduced left ventricular ejection fraction. The most prognostically significant was the TNF-α gene polymorphism: G308A, which was observed in more than 50% of patients. Among the majority of these patients, an increase in plasma TNF-α concentration was detected.

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