



**Original Article**

# Tumor Necrosis Factor-Alpha in Very Elderly Patients with Coronary Artery Disease

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## ABSTRACT

**Background/Purpose:** Limited data are available on tumor necrosis factor-alpha (TNF- $\alpha$ ) in elderly patients with coronary artery disease (CAD), therefore, we evaluated TNF- $\alpha$  levels and their relationships with various disorders.

**Methods:** 130 patients were enrolled in the cross-sectional study: 102 with CAD in the study group, 28 without CAD in the control. Patient's age varied from 77 to 101 years; 56.2% of patients were over 90 years old. Serum TNF- $\alpha$  levels ( $N < 8.1$  pg/ml) were determined by enzyme-linked immunosorbent assay.

**Results:** Mean TNF- $\alpha$  concentration was  $9.2 \pm 4.7$  (3.9-31.9) pg/ml. Increased TNF- $\alpha$  levels were found in 54.6% of patients. In patients with CAD mean TNF- $\alpha$  concentration reached  $10.0 \pm 4.9$  pg/ml, in control group was  $6.1 \pm 1.8$  pg/ml ( $p < 0.001$ ). TNF- $\alpha$  levels were higher in patients with heart failure ( $p = 0.002$ ). Among patients younger than 90 years, much more significant relationships between TNF- $\alpha$  levels and echocardiographic parameters were observed in comparison with patients older than 90 years. In patients with hyperuricemia mean TNF- $\alpha$  concentration was  $10.9 \pm 5.3$  pg/ml, in patients with normal uric acid was  $7.5 \pm 2.5$  pg/ml ( $p < 0.001$ ). In patients with CAD positive correlations were found between TNF- $\alpha$  and uric acid ( $r = 0.45$ ;  $p < 0.001$ ), creatinine ( $r = 0.24$ ;  $p = 0.01$ ), urea ( $r = 0.38$ ;  $p < 0.001$ ), negative correlations were registered between TNF- $\alpha$  and high density lipoprotein (HDL)-cholesterol levels ( $r = -0.42$ ;  $p < 0.001$ ), but there were no significant relationships in the control group. In patients younger than 90 years old mean TNF- $\alpha$  values reached 10.5 pg/ml, in patients older than 90 years was 8.1 pg/ml ( $p = 0.003$ ).

**Conclusion:** The study results demonstrated frequent TNF- $\alpha$  increase in very elderly patients with CAD. Increased TNF- $\alpha$  levels are associated with various pathology.

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## 1. INTRODUCTION

The term "Inflamm-aging", proposed by Franceschi C. et al, refers to the special role of inflammation

in aging processes.<sup>1</sup> This kind of inflammation is described by five main characteristics: mild, controlled, asymptomatic, chronic and systemic. Unlike the usual inflammatory response to any

pathological agent, the inflammation does not disappear, but stably persists, leading to various pathological changes: atherosclerosis, coronary artery disease, type 2 diabetes, osteoporosis, sarcopenia, Alzheimer's disease, Parkinson's disease, oncological and other diseases.<sup>2</sup>

Inflammation is a significant independent risk factor for morbidity and mortality in the elderly. The presence of many diseases associated with inflammation leads to a significant decrease in the functional abilities of the elderly and is associated with the development of frailty syndrome.<sup>3</sup> The correlation between inflammatory processes and age-associated diseases is quite complex and not fully understood.<sup>2</sup> It is believed that a variety of infectious and non-infectious (smoking, obesity, genetic characteristics and gradually decreasing function of sex hormones) factors contribute to systemic inflammation in the elderly.<sup>4</sup> A persistent inflammatory response, tissue damage, and the production of reactive oxygen species lead to an additional release of cytokines, which in turn contributes to the formation of a vicious cycle with further stimulation of immune system remodeling and the development of a chronic pro-inflammatory state.<sup>3</sup>

Of particular importance in the processes of age-related inflammation are pro-inflammatory cytokines. Elevated levels of cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6) are associated with various diseases, disability and mortality in old patients.<sup>5,6</sup> TNF- $\alpha$  and IL-6 are often considered as multifunctional cytokines that have important regulatory properties in immune processes, metabolism of fats, proteins, carbohydrates and in bone metabolism, as well as in the induction of a procoagulant state.<sup>6</sup>

A very important role in the immune response of the elderly is played by such a classic pro-inflammatory cytokine as TNF- $\alpha$ . It has been shown that it is associated with various age-related diseases and, most likely, with an increase in mortality.<sup>4,5,7</sup> TNF- $\alpha$  is known to affect several risk factors for cardiovascular diseases and, in particular, insulin resistance, dyslipidemia, endothelial dysfunction and endothelial activation of cell adhesion molecules. TNF- $\alpha$  causes an increase in basal energy expenditure, anorexia, and loss of muscle and bone mass. The relationship between the TNF- $\alpha$  content and cachexia in chronic inflammatory diseases (including rheumatoid arthritis, cancer, as well as infection with human immunodeficiency virus) has been established. High blood TNF- $\alpha$  levels are associated with lower muscle mass and strength in older men and women.<sup>5</sup>

Conflicting medical literature data served as the basis for our attempt to study the role of subclinical inflammation in various pathologies in very elderly

patients suffering from coronary artery disease. The objectives of this study were: to determine the concentrations of TNF- $\alpha$  in very elderly patients with CAD, to compare TNF- $\alpha$  levels in patients with CAD and without CAD, and to analyze the relationships of TNF- $\alpha$  with a number of various disorders.

## 2. METHODS

The present cross-sectional study enrolled hospitalized men and women  $\geq 75$  years of age with arterial hypertension. The study group included patients older than 75 years suffering from coronary artery disease, the control group included elderly patients with arterial hypertension, but without coronary artery disease. The main exclusion criteria were acute coronary syndrome over the past four weeks, malignant neoplasms in the active phase, any chronic inflammatory diseases, as well as any infectious disease before the enrollment in the study.

Diagnosis of coronary artery disease was based on history of myocardial infarction, percutaneous coronary interventions or coronary artery bypass grafting in the past, as well as on coronary angiography data, resting and exercise electrocardiography findings. The pretest probability of coronary artery disease was assessed.<sup>9</sup> Arterial hypertension has been diagnosed when patient's systolic blood pressure  $\geq 140$  mm Hg and/or diastolic blood pressure  $\geq 90$  mm Hg following repeated examination, or when patient constantly received antihypertensive treatment.

To assess the condition of patients, standard clinical examination methods for CAD were used. The serum TNF- $\alpha$  concentrations were determined by enzyme-linked immunosorbent assay. Serum TNF- $\alpha$  levels were analyzed by commercially available enzyme-linked immunosorbent assay kits (IMMULITE 1000, Siemens, Germany). Reference values of TNF- $\alpha$  levels were less than 8.1 pg/ml. The intra and inter-assay coefficient of variation was 6.0% and 9.3%, respectively. All samples and standards were run as duplicates and the mean of duplicates was used in the statistical analyses. In addition, leptin levels were determined by enzyme-linked immunosorbent assay (Diagnostics Biochem kits, Canada Inc.). IL-6 serum concentrations were analyzed by electrochemiluminescent assay (Elecsys IL-6 kits, Roche Diagnostics GmbH, Switzerland).

Routine laboratory parameters of blood tests and urine tests were also evaluated. Bone mineral density and body composition were analyzed using dual energy X-ray absorptiometry. In addition, a comprehensive geriatric assessment was carried out, including the "Age is not a hindrance" questionnaire, the Barthel index for activities of daily living and the Lawton Instrumental Activities of daily living (IADL) scale.

Ethical approval: All procedures conducted in the study complied with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration. Informed consent was obtained from all participants included in the study. This study was approved by the Independent Review Board of First Moscow State Medical University (Sechenov University); the IRB approval number is 05-2017.

The data were analyzed using Statistica software (version 13.0). To provide the data, descriptive statistics methods were used (mean value and standard deviation for quantitative variables; number and proportion for qualitative variables). The relationships between various indicators were investigated through correlation (using the Spearman test) and regression analysis. The statistical significance of differences between the values was assessed by Mann–Whitney test or chi-square test (as appropriate). Risk for TNF- $\alpha$  increase was also estimated by relative risk (RR) with corresponding two-sided 95% confidence intervals. Differences were considered as statistically significant at  $p < 0.05$ .

### 3. RESULTS

The study enrolled 130 patients. 102 patients suffered from coronary artery disease and made up the study group, 28 patients without coronary artery disease were in the control group. The mean age of patients reached  $89.3 \pm 4.6$  years, varying from 77 to 101 years. More than half of the patients (56.2%) were 90 years old or older; there were only 5 (3.8%) people aged 75 to 80 years. Most patients (65.4%) were women, men accounted for 34.6%. Comparative characteristics of patients with coronary artery disease and without this pathology are presented in Table 1.

All patients enrolled in the study had signs of frailty. The mean value of the “Age is not a hindrance” questionnaire was  $5.1 \pm 0.7$  points, varying from 3 to 7 points. In patients with coronary artery disease and in the control group, the mean scores on the “Age is not a hindrance” scale were identical and amounted to  $5.0 \pm 0.6$  and  $5.1 \pm 0.8$  points, respectively ( $p = 0.5$ ). The mean value of the Lawton Instrumental Activities of daily living (IADL) scale was  $3.8 \pm 2.2$  points, with fluctuations from 0 to 8 points. The mean value of the Barthel index for Activities of Daily Living was  $74.8 \pm 18.3$  points. The patients included in the study received standard therapy for coronary artery disease and arterial hypertension: angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, beta blockers, calcium channels blockers, diuretics, antiplatelet medications. Statins were taken by 13.7% of study patients.

An increased level of serum tumor necrosis factor- $\alpha$  was found in 71 (54.6%) patients. The mean concentration of TNF- $\alpha$  was  $9.2 \pm 4.7$  pg/ml (from 3.9

to 31.9 pg/ml). Among patients with an increased concentration of TNF- $\alpha$ , the mean serum level of this cytokine reached  $12.1 \pm 4.6$  pg/ml, while in patients with a normal level of TNF- $\alpha$  were  $5.8 \pm 1.3$  pg/ml.

Significant differences in the level of TNF- $\alpha$  in men and women were not found. In the group of women, the mean TNF- $\alpha$  concentration was  $9.1 \pm 4.1$  pg/ml, while in men this parameter was  $9.4 \pm 5.7$  pg/ml ( $p = 0.68$ ). In patients under 90 years of age, the mean TNF- $\alpha$  concentration was significantly higher than in patients older than 90 years ( $10.5$  vs  $8.1$  pg/ml;  $p = 0.003$ ). During the correlation analysis, an inverse correlation was found between the level of TNF- $\alpha$  and the age of the patients ( $r = -0.24$ ;  $p = 0.006$ ).

In the group of patients with elevated levels of TNF- $\alpha$ , almost all (95.8%) patients had chronic coronary artery disease, while among patients with normal TNF- $\alpha$ , this disease was registered in 57.6% of cases ( $p < 0.001$ ), Table 2. In patients with coronary artery disease, the mean concentration of TNF- $\alpha$  reached  $10.0 \pm 4.9$  pg/ml,

**Table 1.** Comparative characteristics of patients with and without CAD

Parameter	Study group (CAD) (n=102)	Control group (without CAD) (n=28)	p
Age, years	$89.4 \pm 4.6$	$89.0 \pm 4.8$	0.67
Women, %	65.7%	64.3%	
Men, %	34.3%	35.7%	0.9
Arterial hypertension, %	100%	100%	NS
Myocardial infarction in history, %	45.1%	0	<0.001
Heart failure, %	49.0%	0	<0.001
Atrial fibrillation, %	52.9%	10.7%	<0.001
Stroke in history, %	21.0%	10.7%	0.17
Diabetes mellitus type 2, %	27.7%	33.3%	0.36
Hyperuricemia, %	51.1%	3.7%	<0.001
Obesity, %	47.8%	21.4%	0.01
Body mass index, kg/m <sup>2</sup>	$29.5 \pm 5.1$	$28.2 \pm 4.2$	0.2
Dyslipidemia, %	39.0%	44.4%	0.6
Total cholesterol, mmol/L	$4.3 \pm 1.2$	$5.1 \pm 1.2$	0.004
HDL-cholesterol, mmol/L	$1.17 \pm 0.3$	$1.29 \pm 0.3$	0.10
LDL-cholesterol, mmol/L	$2.6 \pm 0.9$	$3.0 \pm 1.0$	0.04
Triglycerides, mmol/L	$1.39 \pm 1.0$	$1.36 \pm 0.5$	0.85
Glucose, mmol/L	$6.4 \pm 2.7$	$6.6 \pm 2.6$	0.71
Creatinine, $\mu$ mol/L	$109.2 \pm 37.1$	$94.2 \pm 16.5$	0.04
Urea, mmol/L	$8.4 \pm 3.7$	$6.8 \pm 1.4$	0.04
Uric acid, $\mu$ mol/L	$384.6 \pm 139.4$	$283.4 \pm 50.8$	<0.001
Hemoglobin, g/L	$120.2 \pm 19.9$	$121.5 \pm 16.5$	0.76
Erythrocyte sedimentation rate (ESR), mm/hour	$19.7 \pm 13.1$	$16.8 \pm 7.6$	0.26
Leukocytes, $10^9/L$	$5.8 \pm 1.4$	$5.7 \pm 1.3$	0.68
Neutrophil-lymphocyte ratio	$3.1 \pm 1.3$	$3.2 \pm 1.1$	0.72

while in the group of patients without this disorder, the mean TNF- $\alpha$  was  $6.1 \pm 1.8$  pg/ml ( $p < 0.001$ ). 66.6% of patients suffered from CAD had elevated TNF- $\alpha$  levels, while only 10.7% of patients in the control group had elevated concentration of this cytokine ( $p < 0.001$ ). The probability of detecting elevated levels of TNF- $\alpha$  in patients with coronary artery disease increased by 14.6 times, compared with the corresponding parameters in patients without coronary artery disease (Relative Risk (RR)=14.6;  $p < 0.001$ ).

Among patients with congestive heart failure, the mean serum TNF- $\alpha$  values were  $10.8 \pm 5.5$  pg / ml, while in patients without clinically significant heart failure were  $8.1 \pm 3.8$  pg/ml ( $p=0.002$ ). The probability of detecting an increased concentration of TNF- $\alpha$  in patients with heart failure increased by 3.3 times compared with the corresponding parameters in patients without heart failure (RR=3.3;  $p=0.004$ ). In the group of patients with atrial fibrillation, the mean serum TNF- $\alpha$  level reached  $9.98 \pm 5.0$  pg/ml, while in patients without this arrhythmia,  $8.5 \pm 4.4$  pg/ml ( $p=0.1$ ).

Among patients with hyperuricemia, the mean serum TNF- $\alpha$  values were  $10.9 \pm 5.3$  pg/ml, while in patients with normal uric acid levels were  $7.5 \pm 2.5$

pg/ml ( $p < 0.001$ ). During the correlation analysis, a highly significant correlation was established between the serum TNF- $\alpha$  and uric acid levels ( $p < 0.001$ ) in the study group. (Figure 1) However, no significant relationships between TNF- $\alpha$  and uric acid concentrations were found in the control group ( $r=0.05$ ;  $p=0.79$ ).

In the study group of patients, the TNF- $\alpha$  concentrations increased as azotemia increased. The mean creatinine levels in patients with elevated TNF- $\alpha$  reached  $113.6$   $\mu$ mol/L, while in patients with normal TNF- $\alpha$  concentrations were  $96.7$   $\mu$ mol/L ( $p=0.001$ ). The serum urea concentration in patients with elevated TNF- $\alpha$  was also significantly higher ( $8.9$  mmol/L), compared with  $6.9$  mmol/L in the group of patients with normal TNF- $\alpha$  content ( $p < 0.001$ ). Patients with elevated levels of TNF- $\alpha$  showed lower levels of total cholesterol ( $4.2$  compared to  $4.8$  mmol/L in individuals with normal TNF- $\alpha$ ,  $p=0.005$ ) and HDL-cholesterol ( $1.1$  and  $1.3$  mmol/l, respectively,  $p=0.004$ ). Correlation analysis revealed significant relationships between the TNF- $\alpha$  and urea ( $r= 0.38$ ;  $p < 0.001$ ), creatinine ( $r= 0.24$ ;  $p=0.01$ ), HDL-cholesterol ( $r=-0.42$ ;  $p < 0.001$ ) levels in the study group, but in the control group there were no statistically significant correlations ( $p=0.14-0.98$ ).

Significant differences were established between patients with elevated and normal levels of TNF- $\alpha$  in terms of serum interleukin-6 ( $12.9$  and  $7.4$  pg/ml, respectively,  $p=0.02$ ). A correlation analysis found statistically significant direct correlation between the levels of TNF- $\alpha$  and IL-6 ( $r=0.34$ ;  $p=0.01$ ).

In the group of patients with a high concentration of TNF- $\alpha$ , a lower level of serum leptin was registered in comparison with patients with a normal TNF- $\alpha$  content ( $13.2$  and  $18.5$  ng/ml, respectively,  $p=0.05$ ). Also, in the group of patients with an increased TNF- $\alpha$ , higher serum concentrations of collagen degradation products ( $\alpha$ -Cross Laps) were observed ( $0.66$  and  $0.42$  ng/ml, accordingly,  $p=0.01$ ). Correlation analysis revealed the direct correlation between the blood levels of TNF- $\alpha$  and  $\alpha$ -Cross Laps ( $r=0.53$ ;  $p < 0.001$ ), as well as inverse correlation with leptin concentration ( $r=-0.29$ ;  $p=0.01$ ). However, in the control group there were no significant relationships between TNF- $\alpha$  and leptin levels ( $r=0.05$ ;  $p=0.84$ ).

No significant correlations were found between TNF- $\alpha$  values and erythrocyte sedimentation rate ( $19.5$  and  $18.6$  mm/h, respectively,  $p=0.7$ ), as well as hemoglobin level ( $119$  and  $122$  g/L, respectively,  $p=0.31$ ). However, in patients younger than 90 years, a significant inverse correlation was found between the TNF- $\alpha$  concentration and the hemoglobin content, while in patients older than 90 years no significant relationship was found (Table 3).

The mean left atrium diameter in patients with

**Table 2.** Comparative characteristics of patients with increased and normal TNF- $\alpha$  levels

Parameter	Patients with increased TNF- $\alpha$		Patients with normal TNF- $\alpha$		p
	n	%	n	%	
Coronary artery disease	68	95.8	34	57.6	<0.001
Myocardial infarction in history	26	36.6	20	33.9	0.45
Congestive heart failure	35	49.3	16	27.1	0.007
Atrial fibrillation	38	53.5	19	32.2	0.01
Stroke in history	13	18.3	11	18.9	0.57
Diabetes mellitus type II	22	31.4	15	25.9	0.3
Hyperuricemia	34	54.0	14	25.0	0.001

**Figure 1.** Correlations between the serum TNF- $\alpha$  and uric acid levels



elevated TNF- $\alpha$  was 46.2 mm, while with a normal TNF- $\alpha$  levels was 43.8 mm ( $p=0.02$ ). When dilating the left atrium, the mean TNF- $\alpha$  values were  $9.4\pm 4.5$  pg/ml, while among patients with normal left atrium sizes were  $7.7\pm 3.4$  pg/ml ( $p=0.04$ ). In patients with elevated TNF- $\alpha$  levels a significantly higher the pulmonary artery pressure (44.1 mm Hg) was recorded compared to patients with normal TNF- $\alpha$  concentrations (35.8 mmHg,  $p=0.002$ ). With an elevated TNF- $\alpha$  levels, an increase in the right ventricle size was observed (30.9 and 28.9 mm, respectively;  $p < 0.001$ ). Among patients younger than 90 years, the relationships between the TNF- $\alpha$  levels and echocardiographic parameters were much more significant than in patients older than 90 years (Table 4).

No significant relationships were found between the serum TNF- $\alpha$  concentration and all parameters of bone mineral density, both in patients with CAD and in the control group. In addition, no correlations were found between the serum TNF- $\alpha$  levels and the adipose and lean tissue content, both in patients with

CAD and in the control group. However, a significant inverse correlation was found in women between the content of TNF- $\alpha$  and lean tissue in the lower extremities ( $r=-0.44$ ;  $p=0.04$ ), the ratio of adipose tissue in the limbs to trunk fat ( $r=-0.43$ ;  $p=0.04$ ). Also, the correlation between serum TNF- $\alpha$  concentration and the ratio of trunk fat to total fat in women was positive ( $r=0.44$ ;  $p=0.04$ ). Among men, no significant relationships between the concentration of TNF- $\alpha$  and indicators of body composition were found ( $p=0.7-0.98$ ). In obese patients, the inverse correlations between the fat content in the lower extremities ( $r=-0.81$ ;  $p=0.01$ ) as well as the ratio of adipose tissue in the lower extremities to the total fat content ( $r=-0.74$ ;  $p=0.03$ ) and the TNF- $\alpha$  levels were more significant than in patients with normal body weight ( $p=0.7$ ).

No significant relationship was found between the serum TNF- $\alpha$  levels and the severity of frailty ( $r=0.1$ ;  $p=0.4$ ). There was also no correlation between the concentration of TNF- $\alpha$  and the functional abilities of patients (for the Barthel index:  $r=0.03$ ,  $p=0.8$ ; for the IADL scale:  $r=0.01$ ,  $p=0.9$ ). There was no significant correlation between the concentration of TNF- $\alpha$  concentrations and muscle strength according to hand-held dynamometry,  $r=-0.04$ ;  $p=0.76$ .

**Table 3.** Correlations between TNF- $\alpha$  levels and other laboratory parameters in patients <90 years and >90 years old

Parameters	Patients <90 years (n=57)		Patients >90 years (n=73)	
	r	p	r	p
Creatinine	0.34	0.009	0.24	0.04
Urea	0.53	<0.001	0.28	0.02
Uric acid	0.52	<0.001	0.39	0.001
Glucose	-0.21	0.11	0.19	0.10
Total cholesterol	-0.29	0.02	-0.13	0.29
HDL-cholesterol	-0.51	<0.001	-0.22	0.08
LDL-cholesterol	-0.01	0.92	-0.1	0.40
Triglycerides	0.002	0.98	-0.11	0.36
Erythrocyte sedimentation rate	0.22	0.09	-0.12	0.33
Hemoglobin	-0.31	0.02	0.08	0.47
Leukocytes (total)	-0.1	0.41	-0.002	0.98
Neutrophils	-0.12	0.38	-0.06	0.63
Lymphocytes	0.04	0.76	-0.03	0.82
Neutrophil-lymphocyte ratio	-0.21	0.11	-0.04	0.72

**Table 4.** Correlations between TNF- $\alpha$  levels and echocardiographic parameters in patients <90 years and >90 years old

Parameters	Patients <90 years (n=57)		Patients >90 years (n=73)	
	r	p	r	p
Left atrium diameter	0.37	0.006	0.14	0.26
Left ventricle end-diastolic dimension	0.41	0.002	-0.07	0.55
Left ventricle end-systolic dimension	0.41	0.002	-0.14	0.28
Left ventricle end-diastolic volume	0.44	0.001	-0.18	0.15
Left ventricle end-systolic volume	0.46	<0.001	-0.18	0.15
Right ventricle size	0.34	0.01	0.24	0.05
Pulmonary artery pressure	0.32	0.02	0.23	0.07

At the same time in patients younger than 90 years old with a high content of TNF- $\alpha$ , more significant signs of frailty were observed - the mean score for the questionnaire "Age is not a hindrance" was 5.5, and at a normal level of TNF- $\alpha$  was 4.7 points ( $p=0.03$ ). On the contrary, among patients older than 90 years with an increase in the concentration of TNF- $\alpha$ , frailty was less significant than with a normal level of this cytokine, 4.8 and 5.2 points, respectively ( $p=0.07$ ). In patients with coronary artery disease, significant relationships between TNF- $\alpha$  value, frailty and functional abilities of patients were not revealed. However, in the control group in patients with increased TNF- $\alpha$  levels more severe frailty was found (6.3 and 4.9 points;  $p=0.005$ ) as well as significant correlation between the frailty severity and the concentration of this cytokine ( $r=0.57$ ;  $p=0.007$ ).

As a first step of logistic regression analysis, we performed univariate logistic regression screening analysis in order to establish individual associations between TNF- $\alpha$  elevation and various clinical, laboratory and instrumental parameters (Table 5).

All parameters, which were significantly associated with TNF- $\alpha$  elevation based on results of univariate logistic regression analysis, were then included in multivariate logistic regression analysis. Multiple logistic regression analysis revealed following significant factors associated with TNF- $\alpha$  elevation, coronary artery disease ( $p=0.03$ ), IL-6 elevation ( $p=0.02$ ), and right ventricle diameter ( $p=0.01$ ). *P*-value for hyperuricemia was  $p=0.06$ . Other factors lost their significance in multiple logistic regression analysis.

**Table 5.** Univariate logistic regression analysis of TNF- $\alpha$  elevation vs various parameters in total sample

Parameters	Odds Ratio	95% Confidence Interval	p
Coronary artery disease	14.64	4.09–52.44	<0.0001
Congestive heart failure	3.32	1.56–7.09	0.002
Atrial fibrillation	1.93	0.95–3.93	0.07
Age $\geq$ 90 years	0.59	0.29–1.20	0.14
Frailty	2.84	0.28–28.31	0.37
Obesity	1.51	0.72–3.16	0.27
Hemoglobin, g/L	0.21	0.03–1.38	0.10
Neutrophils/lymphocytes index	0.30	0.05–1.82	0.19
ESR, mm/hour	2.03	0.42–9.87	0.37
IL-6 elevation	4.00	1.09–14.66	0.03
Blood urea, mmol/L	8.63	7.67–97.12	0.005
Uric acid, $\mu$ mol/L	3.07	1.42–6.66	0.004
Total cholesterol, mmol/L	0.15	0.03–0.73	0.02
HDL cholesterol, mmol/L	0.02	0–0.24	0.002
LDL cholesterol, mmol/L	0.40	0.08–2.05	0.27
Leptin, ng/mL	0.20	0.02–1.79	0.15
Left atrium diameter, mm	10.43	1.21–89.59	0.03
Left atrium dilation	1.64	0.65–4.15	0.29
Right ventricle diameter, mm	29.35	8.21–104.88	0.002
Pulmonary artery pressure, mmHg	28.69	3.60–228.99	0.001

The mean duration of follow-up of study patients was 21.5 months (0-55 months). During this observation period 60% of patients with elevated TNF- $\alpha$  levels and 22.9% of patients with normal blood TNF levels died ( $p < 0.001$ ). The most significant variables associated with better survival of patients were age over 90 years ( $p=0.01$ ), male sex ( $p=0.001$ ), better functional abilities of patients ( $p=0.002$ ) and less severity of frailty ( $p=0.01$ ), lower levels of TNF- $\alpha$  ( $p=0.001$ ), creatinine ( $p=0.003$ ), urea ( $p=0.0006$ ), total cholesterol ( $p=0.001$ ) and erythrocyte sedimentation rate ( $p=0.01$ ).

#### 4. DISCUSSION

Our results indicate a frequent, but insignificant, increase in the TNF- $\alpha$  levels in very elderly patients. In contrast to the study of Oe Y. and co-authors, who noted a direct correlation with the age of patients, in our study, the TNF- $\alpha$  levels decreased with the aging of patients, which may be due in part to the age differences of study participants.<sup>9</sup> In Japanese work, the mean age of patients was 73 years, and the maximum was 86 years, while our study enrolled older people with a mean age of 89 years and a maximum of 101 years. It can be assumed that in centenarians subclinical inflammation is less significant; therefore, they have fewer diseases and higher life expectancy. However, a study of Bruunsgaard H. et al, who observed 126 patients 100

years of age, found a significant increase in TNF- $\alpha$ , as well as a correlation of this pro-inflammatory cytokine with mortality in patients.<sup>5</sup>

Our study identified a number of significant relationships between increased TNF- $\alpha$  levels and cardiovascular diseases. The most significant association was established between an increase in the TNF- $\alpha$  concentration and the presence of coronary artery disease, as well as clinically significant chronic heart failure. The results of both clinical and experimental studies indicate that TNF- $\alpha$  plays an important role in vascular dysfunction, atherogenesis, arterial hypertension, and pathological myocardial remodeling.<sup>10</sup> An increase in the TNF- $\alpha$  levels in our patients with heart failure is consistent with other studies confirming the role of pro-inflammatory cytokines in the pathogenesis of chronic heart failure, especially with a preserved ejection fraction.<sup>11</sup> It was found, for example, that expression of TNF- $\alpha$  by cardiomyocytes leads to inhibition of their contractile activity. It was also found that TNF- $\alpha$  can interact with  $\alpha$ -adrenergic receptors and thereby exacerbate the negative inotropic effect.<sup>10-12</sup>

According to our results, in the group of patients with atrial fibrillation, higher levels of TNF- $\alpha$  were observed, however, these differences did not reach the statistical significance. These data are consistent with the results of several studies in recent years, which showed that the risk of atrial fibrillation in patients with elevated levels of pro-inflammatory cytokines (including TNF- $\alpha$ ) increases significantly.<sup>14</sup> The specific pathogenetic relationship between proinflammatory cytokines and atrial fibrillation is not yet clear, however, a number of concepts have been proposed that link chronic inflammation with the development and progression of structural and electrophysiological atrial remodeling.<sup>13,14</sup> In our study, as in other works, a significant correlation was found between the TNF- $\alpha$  levels and the diameter of the left atrium.<sup>15</sup>

In the studied group of patients a significant relationship was observed between an increase in the TNF- $\alpha$  levels and hyperuricemia; similar results were obtained by other authors.<sup>16-18</sup> The pro-inflammatory effect of uric acid may be associated with its stimulation of the synthesis of various cytokines (interleukin-1 $\beta$ , interleukin-6 and TNF- $\alpha$ ) by mononuclear cells.<sup>16</sup> Studies on cell culture have also demonstrated the role of uric acid in stimulating apoptosis, which in turn leads to an inflammatory reaction.<sup>19</sup>

In our study, as in other works, a significant negative correlation between TNF- $\alpha$  and high-density lipoprotein-cholesterol levels was demonstrated. It is known that HDL-cholesterol is able to reduce the production of TNF- $\alpha$  by macrophages. Due to the

presence of anti-inflammatory and antioxidant effects, HDL cholesterol can act as a negative regulator of circulating levels of TNF- $\alpha$ .<sup>20</sup>

A somewhat unexpected result of this study is the negative correlation between the TNF- $\alpha$  and total cholesterol levels. As a rule, the results of studies known to us indicate the opposite. For a clinical interpretation of our results, we can propose the following concept. As known, the so-called "cholesterol paradox" is noted in very elderly people and centenarians, the meaning of which is the better survival of people with higher concentrations of total cholesterol, even if they have a cardiovascular pathology.<sup>21</sup> On the contrary, subclinical inflammation and increased TNF- $\alpha$  levels are associated with a worsening prognosis in the elderly. Thus, very elderly with higher total cholesterol but lower proinflammatory cytokines, including TNF- $\alpha$ , are more preserved.

In our study, a direct correlation was found between the TNF- $\alpha$  levels and azotemia, which is consistent with the results of studies by other authors,<sup>22-24</sup> although we did not observe patients with severe impaired renal function. Thus, in a study by Liu C. et al, as significant correlation was found between the level of proinflammatory cytokines, including TNF- $\alpha$ , and the severity of chronic kidney disease and creatinine concentration.<sup>23</sup> It has been shown that elevated levels of TNF- $\alpha$  and other pro-inflammatory mediators contribute to a more rapid decrease in glomerular filtration rate and progression of chronic kidney disease, even taking into account the influence of other factors.<sup>22</sup>

In study group of patients, there was a direct correlation between the serum TNF- $\alpha$  content and the concentration of collagen degradation products ( $\beta$ -Cross Laps). It is known that TNF- $\alpha$  is a key factor stimulating pathological bone resorption in patients with various inflammatory diseases.<sup>25</sup> However, in our study, no correlation was found between the level of TNF- $\alpha$  and bone mineral density. It is possible that in very elderly patients not only stimulation of osteoclasts, but also a decrease in the synthesis and function of osteoblasts play an important role in the state of bone mineral density. The suppression of osteoblast synthesis in such patients was demonstrated by us in another study.<sup>26</sup>

Despite the significant results obtained in this study, there are some limitations to this study. Unlike most similar studies, our work was carried out with the participation of a special population of patients—very elderly patients, suffering from not only clinically significant cardiovascular diseases, but also multiple comorbid pathology that could affect the results of this study. One of the study limitations also related to its cross-sectional rather than prospective nature, and

therefore it was impossible to investigate the causal relationship between TNF- $\alpha$  and various pathology, as well as progression of a number of diseases depending on the level of TNF- $\alpha$  as the patients age.

## 5. CONCLUSION

The study results indicate that in very elderly patients with chronic coronary artery disease an increased serum TNF- $\alpha$  levels are often found. Increased TNF- $\alpha$  concentrations are associated with various pathology. Further studies are needed to investigate the role of TNF- $\alpha$  in subclinical inflammation and the development of various age-related disorders in very elderly individuals and centenarians.

## CONFLICTS OF INTEREST

The researchers claim no conflicts of interest.

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