Subclinical inflammation in very old patients with coronary artery disease

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Abstract. The main objective of this study was to determine the concentrations of tumor necrosis factor-alpha and interleukin-6 and to analyze the relationships of these cytokines with a number of various disorders in very old patients with CAD. 130 very old patients were enrolled in this cross-sectional study: 102 with CAD – in the study group, 28 without CAD – in the control. Serum TNF-α (N<8.1 pg/ml) and IL-6 (N<7.0 pg/ml) levels were determined by enzyme-linked immunosorbent assay. Increased TNF-α levels were found in 54.6%, IL-6 – in 49% of patients. In patients with CAD mean TNF-α concentration reached 10.0$\pm$4.9 pg/ml, in control group – 6.1$\pm$1.8 pg/ml (p<0.001). In patients with CAD mean IL-6 concentration was 10.9 pg/ml, in control group – 5.9 pg/ml (p=0.02). Higher TNF-α and IL-6 levels were found in patients with heart failure (p=0.002 and p=0.04, respectively). In patients with hyperuricemia mean TNF-α concentration was 10.9$\pm$5.3, with normal uric acid – 7.5$\pm$2.5 pg/ml (p<0.001); mean IL-6 values were 10.5$\pm$3.1 and 7.1+3.1 pg/ml, respectively (p=0.001). Positive correlations were found between TNF-α and uric acid (r=0.45; p<0.001), creatinine (r=0.24; p=0.01), urea (r=0.38; p<0.001), negative correlations – between TNF-α and HDL-cholesterol (r=-0.42; p<0.001). Positive correlations were observed between IL-6 and TNF-α (r=0.34; p=0.01), creatinine (r=0.35; p=0.01) and urea (r=0.28; p=0.05). In patients <90 years old mean TNF-α values reached 10.5 pg/ml, in centenarians – 8.1 pg/ml (p=0.003). In patients < 90 years mean IL-6 level was 6.7$\pm$3.2 pg/ml, in centenarians – 9.1$\pm$6.2 pg/ml (p= 0.09).

Keywords: tumour necrosis factor-alfa (TNF-α), ilnterleukin-6 (IL-6), inflammation, coronary artery disease (CAD).

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Introduction

The term "Inflamm-aging", proposed by Franceschi et al. (2000), refers to the special role of inflammation in the aging processes (Franceshi et al., 2000). 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 (Xia et al., 2016).

Inflammation is a significant independent risk factor for morbidity and mortality in older persons. The presence of many diseases associated with inflammation leads to a significant decrease in the functional abilities of older persons and is associated with the development of frailty syndrome (Baylis et al., 2013). The correlation between inflammatory processes and age-associated diseases is quite complex and not fully understood (Xia et al., 2016). 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 older persons (Bruunsgaard et al., 2003). 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 (Baylis et al., 2013).

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-α) and interleukin-6 (IL-6) are associated with various diseases, disability and mortality in old patients (Bruunsgaard & Andersen-Ranberg, 2003; Bruunsgaard & Ladelund, 2003). TNF-α 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 (Bruunsgaard & Ladelund, 2003).

Conflicting medical literature data served as the basis for our attempt to study the role of subclinical inflammation in various pathologies in very old patients suffering from coronary artery disease. ***The main objective of this study*** was to determine the concentrations of tumor necrosis factor-alpha and interleukin-6 and to analyze the relationships of these cytokines with a number of various disorders in very old patients with CAD.

Methodology

This present cross-sectional study enrolled hospitalized men and women ≥ 75 years of age. The study group included patients older than 75 years suffering from coronary artery disease, the control group included old 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.

To assess the condition of patients, standard clinical examination methods for CAD were used. The serum TNF-α (in 130 patients) and IL-6 concentrations (in 50 patients) were determined by enzyme-linked immunosorbent assay. Reference values of TNF-α levels were less than 8.1 pg/ml, IL-6 – less than 7.0 pg/ml. Routine laboratory parameters of blood tests and urine tests were also evaluated. 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.

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). When comparing groups, nonparametric methods were used (Mann-Whitney test, chi-square test or Fisher's exact test); conducted a correlation analysis using the Spearman test.

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 - the control group. The mean age of study patients reached 89.3±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±0.7 points, varying from 3 to 7 points. The mean value of the Lawton Instrumental Activities of daily living (IADL) scale was 3.8±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±18.3 points.

An increased level of serum tumor necrosis factor-alpha was found in 71 (54.6%) patients (Figure 1). The mean concentration of TNF-α was 9.2±4.7 pg/ml (from 3.9 to 31.9 pg/ml). An elevated serum IL-6 levels were found in 49% of patients. The mean IL-6 concentration was 7.96±5.1 pg/ml, ranging from 1.5 to 30.6 pg/ml.

In patients under 90 years of age, the mean TNF-αconcentration was significantly higher than in centenarians (10.5 vs 8.1 pg/ml; $p = 0.003$). During the correlation analysis, an inverse correlation was found between the level of TNF-α and the age of the patients ($r = -0.24$; $p = 0.006$). In persons younger than 90 years, the mean IL-6 level was 6.7±3.2 pg/ml, while in centenarians – 9.1±6.2 pg/ml ($p = 0.09$) (Figure 2).

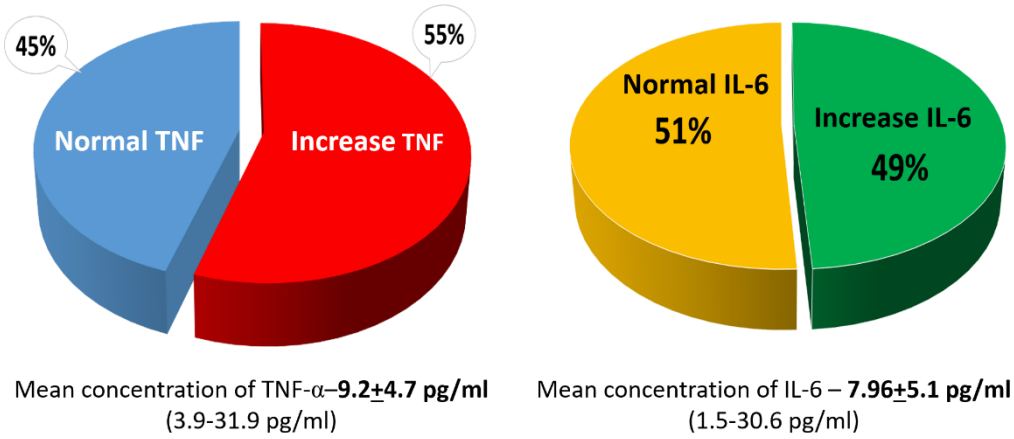
In the group of patients with elevated levels of TNF-α, almost all (95.8%) patients had chronic coronary artery disease, while among patients with normal TNF-α, this disease was registered

in 57.6% of cases (p <0.001) (Figure 3). In the group of patients with an increased IL-6 levels coronary artery disease was diagnosed in 79.2% of cases, while among patients with normal IL-6, this disease was registered in 56% of cases (p = 0.07) (Figure 3).

Table 1: Comparative characteristics of patients with CAD and without CAD

Parameter	**Study group (CAD) (n=102)**	**Control group (without CAD) (n=28)**	**p**
Age, *years*	89.4±4.6	89.0±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	**<0.00001**
Heart failure	49.0%	0	**<0.00001**
Atrial fibrillation	52.9%	10.7%	**<0.00001**
Stroke in history	21.0%	10.7%	0.17
Diabetes mellitus	27.7%	33.3%	0.36
Hyperuricemia	51.1%	3.7%	**<0.00001**
Body mass index, kg/m^2	29.5±5.1	28.2±4.2	0.21

Figure 1: Increase of tumour necrosis factor-α and interleukin-6



Mean concentration of TNF-α–**9.2±4.7 pg/ml** (3.9-31.9 pg/ml)

Mean concentration of IL-6 – **7.96±5.1 pg/ml** (1.5-30.6 pg/ml)

Figure 2: Tumor necrosis factor-α and interleukin-6 in centenarians

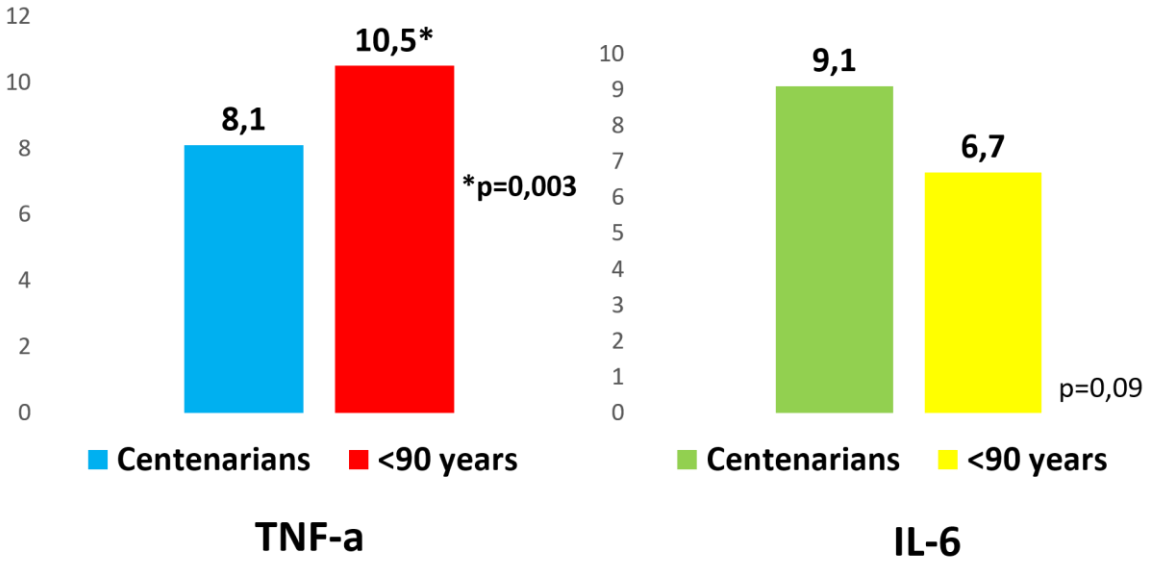
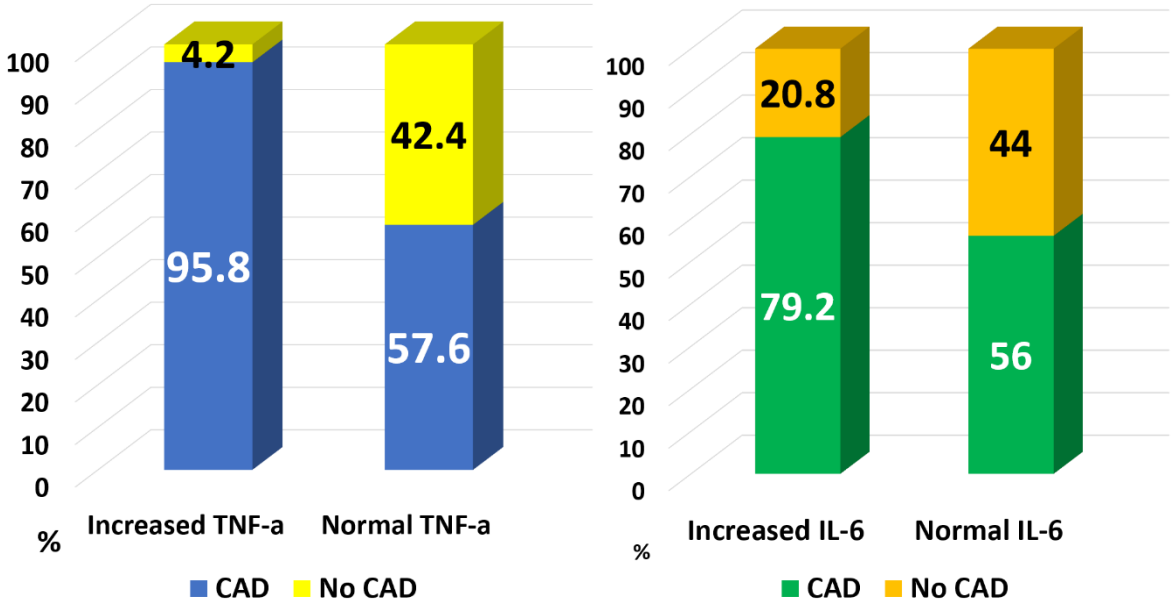


Figure 3: Increase of TNF-α and IL-6 in patients with coronary artery disease

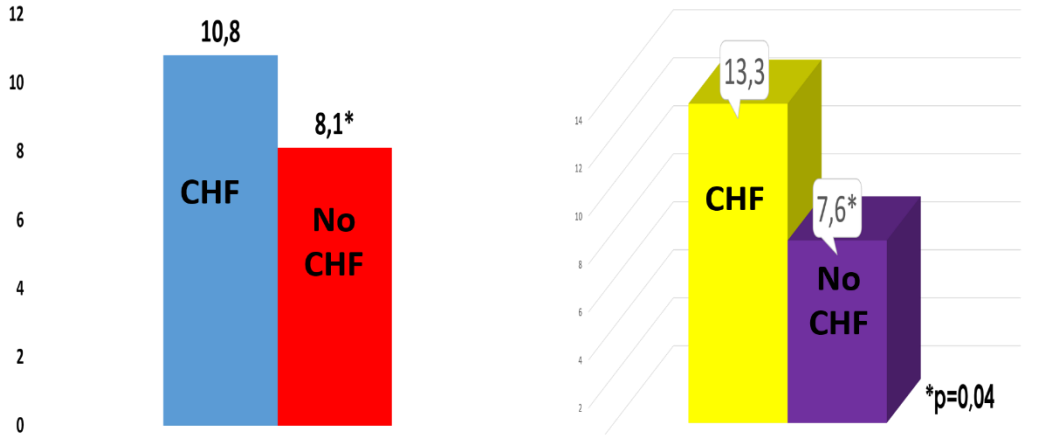


In patients with coronary artery disease, the mean concentration of TNF-α reached 10.0±4.9 pg/ml, while in the group of patients without this disorder, the mean TNF-α was 6.1±1.8 pg/ml ($p < 0.001$). 66.6% of patients suffered from CAD had elevated TNF-α levels, while only 10.7% of patients in the control group had elevated concentration of this cytokine ($p < 0.001$). The probability of detecting elevated levels of TNF-α in patients with coronary artery disease increased by 16.6 times, compared with the corresponding parameters in patients without coronary artery disease (Relative Risk (RR) = 16.6; $p < 0.001$). In patients with coronary artery

disease, the mean interleukin-6 concentration reached 10.9 pg/ml, whereas in the group of patients without coronary artery disease, the mean IL-6 was 5.9 pg/ml (p = 0.02).

Among patients with congestive heart failure, the mean serum TNF-α values were 10.8±5.5 pg / ml, while in patients without clinically significant heart failure – 8.1±3.8 pg/ml (p =0.002) (Figure 4). The probability of detecting an increased concentration of TNF-α in patients with heart failure increased by 2.8 times compared with the corresponding parameters in patients without heart failure (Relative Risk (RR) = 2.8; p = 0.004). Clinically significant chronic heart failure occurred significantly more often among patients with a high serum IL-6 concentration compared with patients who had a normal content of IL-6 (45.8% and 16% of cases, respectively). Significantly higher serum IL-6 levels were registered in the group of patients with heart failure compared with patients without heart failure (Figure 4). The probability of detecting an increased level of IL-6 with clinically significant heart failure increased by 4.4 times, compared with the corresponding indicators in patients without heart failure (Odds ratio (OR) = 4.4; 95% CI = 1.2-16.9; p = 0.02).

Figure 4: Tumor necrosis factor-α and interleukin-6 in patients with heart failure



Odds Ratio for increase TNF-α=2.8; 95% CI=1.3-6.0; **p=0.004**

Odds Ratio for increase IL-6=4.4; 95% CI=1.2-6.9; **p=0.02**

Among patients with hyperuricemia, the mean serum TNF-α values were 10.9±5.3 pg/ml, while in patients with normal uric acid levels – 7.5±2.5 pg/ml (p <0.001) (Figure 5). During the correlation analysis, a highly significant correlation was established between the serum TNF-α and uric acid levels (p <0.001) (Figure 6). Also, the mean serum values of IL-6 were 10.5±3.1 pg/ml among patients with hyperuricemia, while in patients with normal uric acid levels – 7.1±3.1 pg/ml (p = 0.01) (Figure 5). The probability of detecting an increased level of IL-6 with hyperuricemia increased by 9.7 times, compared with the corresponding parameters in patients with normal uric acid concentration (OR = 9.7; 95% CI = 1.9-20.8; p = 0.003). Correlation analysis revealed a significant positive correlation between the IL-6 and serum uric acid levels (R = 0.31; p = 0.03). In a regression analysis, IL-6 (β = 0.46; p = 0.00001) and creatinine (β = 0.64; p<0.000001) were the most important variables for the uric acid

concentrations. The most important variable for the IL-6 content was hyperuricemia (β = 0.7; p = 0.00003).

In the study group of patients, the TNF-α concentrations increased as azotemia increased. The mean creatinine levels in patients with elevated TNF-α reached 113.6 μmol/L, while in patients with normal TNF-α concentrations – 96.7 μmol/L (p = 0.001). The serum urea concentration in patients with elevated TNF-α was also significantly higher – 8.9 mmol/L, compared with 6.9 mmol/L in the group of patients with normal TNF-α content (p <0.001). Patients with elevated levels of TNF-α showed lower levels of total cholesterol (4.2 compared to 4.8 mmol/L in individuals with normal TNF-α, 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-α and urea (r= 0.38; p <0.001), creatinine (r= 0.24; p= 0.01), HDL-cholesterol (r= -0.42; p <0.001) levels.

No significant correlations were found between TNF-α 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-α concentration and the hemoglobin content, while in the centenarians no significant relationship was found (Table 2). Significant differences were established between patients with elevated and normal levels of TNF-α 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-α and interleukin-6 (r = 0.34; p = 0.01).

Figure 5: Tumour necrosis factor-α and interleukin-6 in patients with hyperuricemia

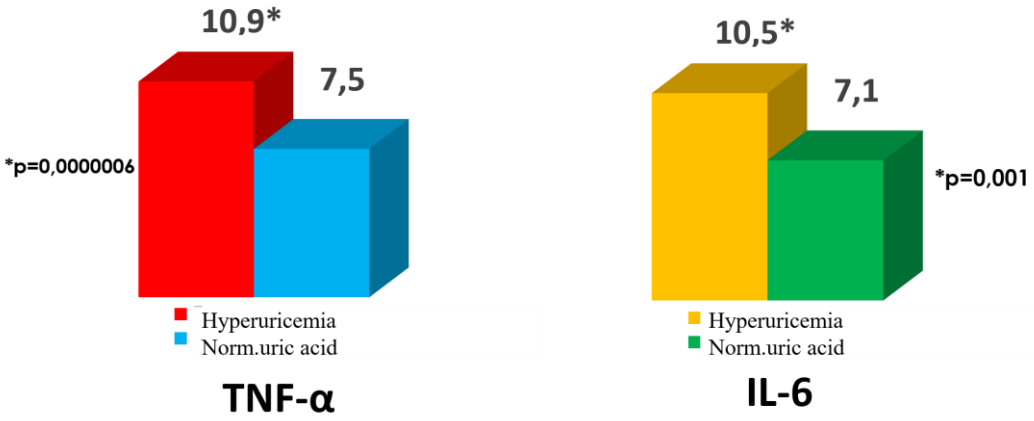


Figure 6: Correlations between tumour necrosis factor-α and serum uric acid

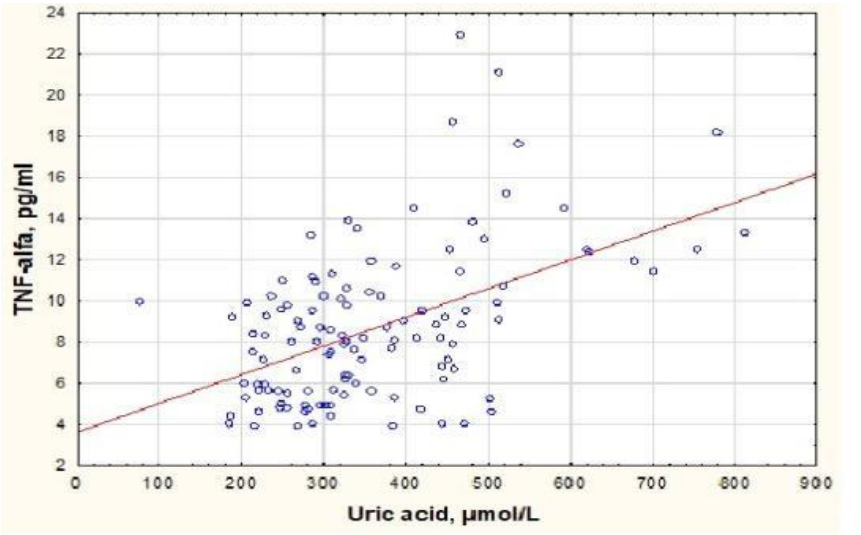


Table 2: Correlations between TNF-α levels and other laboratory parameters in centenarians and in patients <90 years old

Parameters	<90 years (n=57)		Centenarians (n=73)	
	r	p	r	p
Creatinine	0.34	**0.009**	0.24	**0.04**
Urea	0.53	**0.0001**	0.28	**0.02**
Uric acid	0.52	**0.00005**	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	**0.0003**	-0.22	0.08
LDL-cholesterol	-0.01	0.92	-0.1	0.40
ESR	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

In the study group of patients, there was an increase in the IL-6 levels with increasing azotemia: 7.3 pg/ml (with normal renal function) and 14.3 pg/ml in patients with azotemia (p = 0.04). The mean creatinine levels in patients with elevated IL-6 were 105.6 μmol/L, while in patients with normal IL-6 levels – 96.0 μmol/L (p = 0.09). Correlation analysis revealed a significant positive between the levels of IL-6 and creatinine. In women, the relationships between the concentrations of IL-6 and creatinine (r = 0.52; p = 0.002), as well as urea (r = 0.37;

p = 0.03) were much more significant than in men (r = 0,14, p = 0.57 - for creatinine; r = 0.1, p = 0.68 - for urea). Among the centenarians, neither the correlation with creatinine (r = 0.26; p = 0.2), nor with urea (r = 0.1; p = 0.6) were not significant.

There were no significant relationships between the serum levels of IL-6 and lipids, glucose, erythrocyte sedimentation rate, hemoglobin. It should be noted that a significant positive correlation between the IL-6 levels and erythrocytes sedimentation rate (r = 0.75; p = 0.0007) was found in men, as well as a tendency toward an inverse correlation between the content of IL-6 and hemoglobin (r = -0.36; p = 0.1). However, no significant relationships between these parameters were found in women (p = 0.95 and p = 0.71, respectively). Centenarians revealed a positive correlation between IL-6 and ESR (r = 0.51; p = 0.01), along with a tendency to inverse correlation with hemoglobin (r = -0.29; p = 0.1); while in persons under 90 years of age, no significant relationships were found (p = 0.92 and p = 0.58, respectively). There were no significant relationships between the IL-6 levels and leukocyte subpopulations, although a positive correlation was established between IL-6 concentration and the total leukocyte content (r = 0.27; p = 0.05) and a tendency to a similar correlation with the neutrophils content (r = 0.22; p = 0.1). In centenarians, significant positive correlations were found between the IL-6 levels and the total content of leukocytes (r = 0.51; p = 0.008), as well as neutrophils (r = 0.43; p = 0.02); in persons under 90 years of age, no significant relationships were noted (p = 0.67).

The mean left atrium diameter in patients with elevated TNF-α was 46.2 mm, while with a normal TNF-α levels – 43.8 mm (p = 0.02). When dilating the left atrium, the mean TNF-α values were 9.4$\pm$4.5 pg/ml, while among patients with normal left atrium sizes - 7.7$\pm$3.4 pg/ml (p = 0.04). In patients with elevated TNF-α levels a significantly higher the pulmonary artery pressure (44.1 mm Hg) was recorded compared to patients with normal TNF-α concentrations (35.8 mmHg, p = 0.002). With an elevated TNF-α 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-α levels and echocardiographic parameters were much more significant than in centenarians (Table 3).

Table 3: Correlations between TNF-α levels and echocardiographic parameters in centenarians and in patients <90 years old

Parameters	Patients<90 years (n=57)		Centenarians (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

No significant relationship was found between the serum TNF-α levels and the severity of frailty ($r = 0.1$; $p = 0.4$). There was also no correlation between the concentration of TNF-α 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-α concentrations and muscle strength (according to hand-held dynamometry) ($r = -0.04$; $p = 0.76$). At the same time in patients younger than 90 years old with a high content of TNF-α, 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-α– 4.7 points ($p = 0.03$). On the contrary, among centenarians with an increase in the concentration of TNF-α, frailty was less significant than with a normal level of this cytokine – 4.8 and 5.2 points, respectively ($p = 0.07$).

Also, no significant relationship was found between the serum IL-6 concentrations and the severity of frailty. No significant correlations were found between the IL-6 levels and muscle strength ($r = 0.15$; $p = 0.33$), as well as the functional abilities of patients. In centenarians, there is a tendency to a correlation between the IL-6 levels and muscle strength ($r = 0.3$; $p = 0.1$), but in patients younger than 90 years no relationship was found between these parameters ($r = -0.01$; $p = 0.96$). There were also no significant differences in the severity of frailty and functional abilities in patients with elevated and normal serum levels of IL-6.

Conclusion

The study results indicate that in very old patients with chronic coronary artery disease an increased serum TNF-α and L-6 levels are often found. Higher TNF-α and IL-6 concentrations are associated with the chronic heart failure and hyperuricemia. Despite the significant results obtained in this study, there are some limitations to this study. Unlike most similar studies, this study was carried out with the participation of a special population of patients – very old 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 could also be related to its cross-sectional rather than prospective nature, and therefore making it impossible to investigate the progression of a number of diseases depending on the level of TNF-α or IL-6 as the patients age. Further studies are needed to investigate the role of TNF-α and IL-6 in subclinical inflammation and the development of various age-related disorders in very old individuals and centenarians.

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