Connective tissue growth factor in very old patients with coronary artery disease

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Abstract. The main objective of this study was to determine the concentration of connective tissue growth factor in the blood of CAD patients in old age and to establish its clinical significance in various pathologies. This cross-sectional study enrolled patients suffering from coronary artery disease (CAD) with mean age of 87.8 years (75-96 years). The control group consisted of 12 healthy young people (mean age-22.9 years). The blood concentration of connective tissue growth factor (CTGF) was determined by enzyme-linked immunosorbent assay. The mean concentration of CTGF in CAD patients was 357.2 pg / ml, in healthy individuals - 1076.7 pg / ml ($p = 0.07$). In patients with congestive heart failure CTGF concentration was significantly higher than in patients without heart failure ($p = 0.001$). Negative correlation was registered between CTGF levels and systolic ($r = -0.25$; $p = 0.1$) and diastolic ($r = -0.36$; $p = 0.02$) blood pressure. In patients with pneumosclerosis median CTGF concentration reached 190.7 pg/l, without it - 34.7 pg/ml ($p = 0.03$). Significant inverse correlation was found between CTGF and glucose ($r = -0.34$; $p = 0.03$), total cholesterol ($r = -0.49$; $p = 0.002$) and LDL cholesterol ($r = -0.40$; $p = 0.01$) concentrations.

Keywords: connective tissue growth factor (CTGF); fibrosis; coronary artery disease; older persons.

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Introduction

Connective tissue growth factor (CTGF, CCN2) is a small, cysteine-rich extracellular matrix protein. CTGF regulates a variety of cellular functions, including proliferation, migration, adhesion, differentiation and synthesis of extracellular matrix proteins in cells of various types, and also participates in more complex biological processes of angiogenesis, chondrogenesis, osteogenesis, wound healing, fibrosis and oncogenesis. Increased expression of CTGF is observed, notably, in pathological conditions associated with fibrosis (Arnott et al., 2011; Ponticos, 2013).

CTGF plays a role in some pathological processes in the cardiovascular system, including heart failure, cardiosclerosis and scarring after myocardial infarction (Ponticos, 2013). Increased vascular CTGF expression is associated with atherogenesis, apoptosis of smooth muscle cells and the formation of vascular aneurysms, as well as aortic dissection or rupture (Cicha et al., 2005; Cicha et al. 2006; Meng et al., 2014; Sachdeva et. al., 2017). CTGF can participate in the development of cerebral microbleeds induced by arterial hypertension due to the violation of the integrity of the vascular wall (Ungvari et al., 2017). CTGF plays a role in regulating the stability of atherosclerotic plaques and can stimulate the migration of monocytes in them (Ponticos, 2013). In one of the largest clinical studies of CTGF involving 1227 patients with cardiovascular pathology, it was found that an increased level of this factor in the blood increases the risk of new cardiovascular diseases and mortality from all causes (Gerritsen et al., 2016).

It was also found that CTGF acts as an important regulator of skeletogenesis. Correct regulation of CTGF expression is necessary for the normal course of the processes of mesenchymal condensation, chondrogenesis and osteogenesis (Arnott et al., 2011). In addition, CTGF is actively involved in the formation of cartilage. CTGF significantly increases the production of cartilage matrix proteins, and also stimulates the proliferation and differentiation of chondrocytes (Kubota & Takigawa, 2014). CTGF increases, in addition, the adhesion of chondrocytes to fibronectin and angiogenesis (Itoh et al., 2013). The results of experimental studies indicate that CTGF is a key regulator of the formation of the extracellular cartilage matrix. The content of CTGF also increases markedly in numerous pathological conditions accompanied by fibrosis, in which excessive collagen production is stimulated. It has been shown that the content of CTGF is significantly reduced in dermal fibroblasts (the main collagen-producing cells) of people over 80 years old. In contrast, CTGF overexpression stimulated the synthesis of type I procollagen (Quan et al., 2010).

Experimental data indicate that aging is associated with increased expression of CTGF both in blood vessels and in the heart, which may contribute to age-related remodeling of the extracellular matrix (Ungvari et al., 2017). By decreasing the expression of some types of microRNA, CTGF is involved in age-related changes in cardiomyocytes and vascular wall cells (Ungvari et al., 2017). An increased expression of CTGF was also found in "aging" fibroblasts (Jun & Lau, 2017). In connection with these data, CTGF is sometimes considered as a possible marker of aging processes. However, there are practically no clinical studies of CTGF in older people and centenarians. The sporadic and conflicting data published in the

medical literature served as the basis for our attempt to study the growth factor of connective tissue in older patients.

The main objective of this study is to determine the concentration of connective tissue growth factor in the blood of CAD patients in old age and to establish its clinical significance in various pathologies in this group of patients.

Methodology

This work was a cross-sectional study performed at the clinical base of the Hospital for War Veterans No. 3 (Moscow). The study enrolled 50 people; 38 of them suffered from coronary artery disease and constituted the main group, and 12 healthy young people (on average 22.9 years) without coronary artery disease entered the control group. 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. In the absence of the above criteria, the CAD diagnosis was confirmed on the basis of the diagnostic algorithm proposed by the European Society of Cardiology. The pre-test probability of coronary artery disease was assessed.

To assess the condition of patients, standard methods of examination of patients with coronary artery disease, as well as echocardiography, were used. The body weight and height of the patients were determined, and the Body Mass Index (BMI) was calculated using the formula Weight (kg) / Height $(m)^2$. 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 (ADL) and the Lawton Instrumental Activities of daily living (IADL) scale, the Timed Up and Go Test.

The concentration of connective tissue growth factor in the serum was determined by enzyme-linked immunosorbent assay. We used test systems manufactured by BCM Diagnostics, supplier of BioChemMak. The range of normal values for this growth factor has not yet been established; the range of possible measurements ranged from 62.5 to 4000 pg / ml. All samples and standards were run as duplicates and the mean of duplicates was used in the statistical analyses. In addition, standard laboratory parameters of blood and urine tests were assessed. The bone mineral density (BMD) of the lumbar spine and the proximal femur was also measured by dual-energy X-ray absorptiometry on a Lunar Prodigy Advance (GE) apparatus. During the examination, the absolute value of BMD (in g / cm^2) and the T-score (deviation of BMD from the value of peak bone mass at a young age) were determined. For the diagnosis of osteoporosis and osteopenia, the WHO criteria were used, according to which BMD is assessed by the T-score: 1 – normal (BMD values decreased by no more than 1SD); 2 – osteopenia (decrease in BMD by more than 1SD, but not reaching –2.5SD); 3 – osteoporosis (decrease in BMD by -2.5 SD or more).

The data obtained were analyzed using Statistica software (version 13.0). The sample for belonging to the normal distribution was checked using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics methods were used to describe the data obtained

(mean values, standard deviation, minimum, maximum – for quantitative variables; number and proportion – for qualitative variables). In the case when the distribution of variation series did not meet the criteria of "normality", the methods of nonparametric statistics were used, the median (Me), quartiles (Q1-Q4) and interquartile range (from 25% to 75%) were determined. When comparing groups, nonparametric methods were used (Mann-Whitney or Kruskal-Wallis test, chi-square test or Fisher's exact test); correlation analysis was performed using Spearman's test.

Results

Clinical and demographic characteristics of older patients included in the study are presented in Table 1. As can be seen from this table, 71% of patients were women and 29% were men. On average, the age of patients in the group reached 87.8 ± 5.1 years (varying from 75 to 96 years); more than half of the patients (52.6%) were over 90 years old. In addition to coronary artery disease and arterial hypertension, the patients included in the study had a high level of comorbidity (Table 1).

Table 1: Clinical and demographic characteristics of study patients

Parameters	**Number of patients**	
	n	%
Gender		
Men	11	29.0
Women	27	71.0
Age, *years*		
75-80 years	5	13.2
81-89 years	13	34.2
≥ 90 years	20	52.6
Myocardial infarction (in history)	12	31.6
Congestive heart failure	9	23.7
Atrial fibrillation	15	39.5
Stroke (in history)	6	15.8
Diabetes mellitus type II	15	39.5
Arterial hypertension	38	100

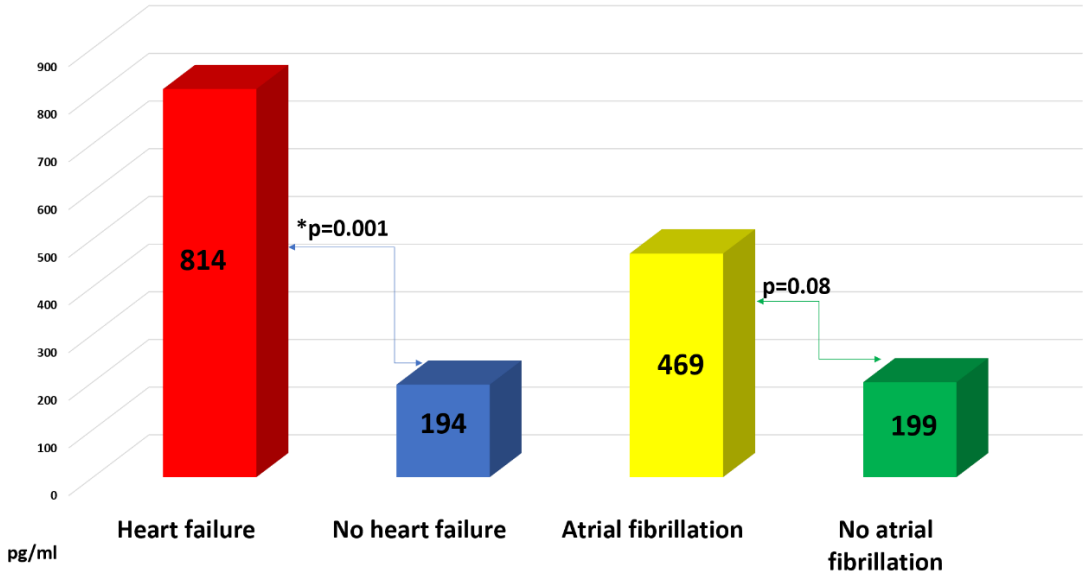
All the patients had signs of frailty. The mean value of the questionnaire "Age is not a hindrance" was 4.2±0.9 points, varying from 3 to 6 points. The mean value of the scale of instrumental activity in everyday life (IADL) was 4.5±2.5 points. The mean value of the scale of basic activity in everyday life (ADL, Bartel's index) reached 79.6±20.9 points.

In the group of older patients with coronary artery disease, the mean concentration of CTGF was 357.2 pg / ml, in the group of healthy individuals - 1076.7 pg / ml (p = 0.07). The median of CTGF in patients with coronary artery disease was 168.3 pg / ml, only 7.9% in this group

had CTGF levels exceeding 1000 pg / ml. In the group of healthy individuals, the content of CTGF exceeded 1000 pg / ml in 25% of cases (p = 0.1 - compared with patients with coronary artery disease). No significant differences in CTGF levels were found between men and women. The median blood concentration of CTGF in women was 154.2 pg / ml, while in men this value was 182.4 pg / ml (p = 0.17). In the course of the correlation analysis, no significant relationships were found between the CTGF levels and the age of patients (r = -0.03; p = 0.86). Among patients with myocardial infarction in history, the median CTGF concentration reached 186.5 pg / ml, while in the group of patients without myocardial infarction, this value was 124.3 pg / ml (p = 0.5). In the group of patients who had myocardial infarction in the past, only 8.3% of patients had the lowest (corresponding to 1 quartile, Q1) values of CTGF, while in patients without myocardial infarction, this indicator was 34.6%.

In the group of patients with clinically significant congestive heart failure (NYHA functional class III-IV), the level of CTGF in the blood was significantly higher than in patients without severe heart failure (p = 0.001) (Figure 1). Among patients without clinically significant signs of heart failure, the highest CTGF values (corresponding to Q4) were recorded in 14.3% of cases, while in patients with severe CHF - in half (50%) of cases. Similar results were obtained with atrial fibrillation, however, the differences between patients with this arrhythmia and without cardiac arrhythmias did not reach the statistical significance (p = 0.08) (Figure 1). In the group of patients with sinus rhythm, the highest CTGF values (corresponding to Q4) were registered in 13.6% of cases, while in patients with atrial fibrillation - in a third (33.3%) of cases.

Figure 1: Connective tissue growth factor & Heart failure and Atrial fibrillation



In our observations, negative correlations were found between CTGF blood levels and values of systoic (r = -0.25; p = 0.1) and diastolic (r = -0.36; p = 0.02) blood pressure. In patients whose CTGF concentration corresponded to 1 quartile (Q1), the mean level of systolic blood pressure was 146.5 mm Hg, while in the 4th quartile (Q4) – 130.5 mm Hg. (p = 0.06). Diastolic blood

pressure reached 85 mm Hg. and 73.3 mm Hg. (Q1 and Q4, respectively; p = 0.007). The blood level of CTGF, depending on the presence of comorbid conditions, is presented in Table 2.

Table 2 Connective tissue growth factors and various diseases

Diseases	**CTGF,** *pg/ml* [Me (Q25; Q75)]*		
	"+" Disease	**"-" Disease**	**p**
Diabetes mellitus type II	69.1 (24.5; 214.9)	206.7 (41.7; 453.4)	0.2
Obesity	69.1 (15.2; 214.9)	206.7 (65.3; 553.6)	*0.05*
Hyperuricemia	215.3 (12.5; 487.1)	186.5 (57.6; 441.4)	0.6
Stroke (in history)	207.1 (69.1; 383.4)	168.3 (34.8; 441.5)	0.9
Pneumosclerosis	190.7 (65.3; 415.4)	34.7 (19.3; 38.9)	**0.03**
Osteoporosis	190.7 (15.2; 429.5)	65.3 (36.2; 406.1)	0.7
Osteoarthritis	172.5 (24.5; 453.4)	182.4 (65.3; 456.1)	0.8

* *Me – median, Q25 and Q75 – 25% and 75% quartiles, respectively.*

As can be seen from the above table, there were no significant differences in the blood concentration of CTGF in the group of patients with diabetes mellitus and without disorders of carbohydrate metabolism. In obese patients, the concentration of CTGF was lower than in patients with normal body weight, however, correlation analysis did not reveal significant relationships between the level of CTGF and body mass index values (r = -0.24; p = 0.15). Among patients with signs of pneumosclerosis (according to computed tomography or chest x-ray), high CTGF values (corresponding to Q3-Q4) were registered in 55% of cases, while in patients without obvious signs of pneumosclerosis, CTGF values in Q3-Q4 did not occur at all. In the group of patients with diagnosed (according to the densitometric study) osteoporosis, high CTGF (Q3-Q4) values were found in 53% of cases, while in patients without osteoporosis, the values of this growth factor corresponding to Q3-Q4 were recorded in 36% of cases. The relationships between the concentration of CTGF and other laboratory parameters are shown in Table 3. The results of the correlation analysis indicate the significant inverse relationship between the level of CTGF and glucose, as well as total cholesterol and LDL cholesterol (Table 4).

During the analysis of relationships between the CTGF concentration and echocardiographic parameters in the total group of patients, no significant correlations were found: for the left ventricle ejection fraction – r = -0.24, p = 0.15; for the diameter of the left atrium – r = 0.14; p = 0.41; for the end-diastolic dimension of the left ventricle – r = -0.14; p = 0.41; for the diameter of the right ventricle – r = 0.18; p = 0.29. However, in the subgroup of patients with congestive heart failure, strong direct correlation was registered between the CTGF level and the diameter of the right ventricle (r = 0.74; p = 0.02), as well as the left atrium (r = 0.51; p = 0.07). In regression analysis the CTGF level turned out to be one of the significant factors associated with the diameter of the right ventricle (β = 0.58; p = 0.004), the calculated pressure in the pulmonary artery (β = 0.62; p = 0.00006) and the diameter of the left atrium (β = 0.46; p = 0.045). Correlation analysis did not reveal any significant relationships between the blood CTGF concentration and all indicators of bone mineral density: r = -0.27, p = 0.17 – for the correlation between CTGF and BMD values in the lumbar spine, r = -0.26, p = 0.19 – for the correlation between CTGF and BMD values in the proximal femur. It should be noted that in women

Table 3: CTGF and other laboratory parameters

Parameters	CTGF			p
	Q1*	Q2-Q3*	Q4*	
Creatinine, *μmol/L*	99.2	105.3	99.2	p=1.0–for differences between group 1 and 3 p=0.6–between group 1 and 2
Urea, *mmol/L*	7.9	7.8	7.4	p=0.7– for differences between group 1 and 3 p=0.9–between group 1 and 2
Glomerular filtration rate, *ml/min*	46.5	47.9	46.3	p=0.9– for differences between group 1 and 3 p=0.8–between group 1 and 2
Uric acid, *μmol/L*	354.2	288.9	300.4	p=0.3– for differences between group 1 and 3 p=0.09–between group 1 and 2
Glucose, *mmol/L*	8.0	6.7	6.8	p=0.3– for differences between group 1 and 3 p=0.2–between group 1 and 2
Total cholesterol, *mmol/L*	5.6	4.7	3.8	**p=0.0009**–for differences between group 1 and 3 **p=0.02**–between group 1 and 2
HDL cholesterol, *mmol/L*	1.2	1.4	1.1	p=0.5– for differences between group 1 and 3 p=0.3–between group 1 and 2
LDL cholesterol, *mmol/L*	3.5	2.7	2.2	**p=0.001**–for differences between group 1 and 3 **p=0.02**–between group 1 and 2
Triglycerides, *mmol/L*	1.7	1.4	1.1	*p=0.05*– for differences between group 1 and 3 p=0.4–between group 1 and 2
Atherogenic index	3.6	2.6	24	**p=0.01**– for differences between group 1 and 3 **p=0.02**–between group 1 and 2

*- *Q1-Q4 – quartiles*

Table 4 : Correlations between CTGF levels and other laboratory parameters

Parameters	r	p
Creatinine	0.17	0.29
Urea	-0.001	0..9
Glomerular filtration rate	-0.14	0,42
Uric acid	-0.03	0.85
Glucose	-0.34	**0.03**
Total cholesterol	-0.49	**0.002**
HDL cholesterol	-0.13	0.4
LDL cholesterol	-0.40	**0.01**
Triglycerides	-0.20	0.23
Atherogenic index	-0.25	0.13
Urine protein	-0.06	0.7
Urine erythrocytes	0.07	0.6

there was the tendency for inverse correlation between BMD values and the CTGF level, which, however, did not reach the statistical significance (r = -0.31; p = 0.1).

Significant relationships between the blood concentration of CTGF and the severity of frailty were not found: r = -0.18, p = 0.3 – for the correlation between CTGF and the values of the questionnaire "Age is not a hindrance." There was also no correlation between the

concentration of CTGF and functional abilities of patients: r = 0.18; p = 0.3 – for the correlation between CTGF and the values of the Activities of Daily Living scale; r = -0.04, p = 0.8 – for the correlation between CTGF and the values of the Instrumental Activities of Daily Living scale.

Conclusion

Within the framework of this pilot study, in older patients with coronary artery disease, significant effect of connective tissue growth factor on echocardiographic indicators of myocardial dysfunction and the course of chronic heart failure was revealed, which indirectly confirms the possible role of this growth factor in the development and progression of heart failure. There was also tendency towards a higher content of CTGF in patients with atrial fibrillation, indicating the possible participation of this factor in the processes of fibrosis and atrial remodeling. At the same time, significantly higher content of CTGF was found in patients with pneumosclerosis, which may indirectly indicate significant role of this factor in the development and progression of pulmonary fibrosis. In addition, significant inverse correlations were revealed between the concentration of CTGF in the blood and indicators of lipid and carbohydrate metabolism, which, however, require additional studies to establish specific pathogenetic relationships between CTGF and various metabolic disorders. A small sample of patients and extremely variable CTGF values do not allow at the moment to draw unambiguous conclusions about the role of this growth factor in various comorbid conditions. Further studies are required to study the role of connective tissue growth factor in aging processes and the development of a number of age-associated diseases in older people.

References

Arnott, J.A, Lambi, A. G, Mundy, C., Hendesi, H, Pixley, R. A., Owen, T. A. Safadi, F. F., & Popoff, S. N. (2011). The role of connective tissue growth factor (CTGF/CCN2) in skeletogenesis. *Critical Reviews in Eukaryotic Gene Expression, 21*(1), 43–69. https://doi.org/10.1615/critreveukargeneexpr.v21.i1.40

Cicha, I., Yilmaz, A., Klein, M., Raitghel, D., Brigstock, D.R., Daniel, W.G., Goppelt-Struebe, M., & Garlichs. C.D.(2005). Connective tissue growth factor is overexpressed in complicated atherosclerotic plaques and induces mononuclear cell chemotaxis in vitro. *Arteriosclerosis, thrombosis, and vascular biology, 25*(5), 1008-1013. https://doi.org/10.1161/01.ATV.0000162173.27682.7b.

Cicha, I., Yilmaz, A., Suzuki, Y., Maeda, N., Daniel, W.G., Goppelt-Struebe, M., & Garlichs, C.D. (2006). Connective tissue growth factor is released from platelets under high shear stress and is differentially expressed in endothelium along atherosclerotic plaques. *Clinical hemorheology and microcirculation, 35*(1-2), 203-206.

Gerritsen, K.G, Falke, L.L, & van Vuuren, S.H Leeuwis, J.W., Broekhuizen, R., Nguyen, T.Q., de Borst, G.J., Nathoe, H.M., Verhaar, M.C., Kok, R.J., Goldschmeding, R., Visseren, & SMART Study Group (2016). Plasma CTGF is independently related to an increased risk of cardiovascular events and mortality in patients with atherosclerotic disease: the SMART study. *Growth Factors, 34*(3–4), 149–158. https://doi.org/ 10.1080/08977194.2016.1210142

Itoh, S., Hattori, T., Tomita, N., Aoyama, E., Yutani, Y., Yamashiro, T., & Takigawa, M. (2013). CCN family member 2/Connective Tissue Growth Factor (CCN2/CTGF) has anti-aging effects that protect articular cartilage from age-related degenerative changes. *PLoS ONE, 8*(8), 1–31. https://doi.org/ doi: 10.1371/journal.pone.0071156 6.

Jun, J.I.I., & Lau, L.F. (2017). CCN2 induces cellular senescence in fibroblasts. *Journal of Cell Communication and Signalling, 11*(1), 15–23. http://doi.org/ 10.1007/s12079-016-0359-1

Kubota, S., & Takigawa, M. (2015). Cellular and molecular actions of CCN2/CTGF and its role under physiological and pathological conditions. *Clinical Science (London), 128*(3), 181–196. https://doi.org/10.1042/CS20140264

Meng, Y., Tian, C., Liu, L., Wang, L., &Chang, Q. (2014). Elevated expression of connective tissue growth factor, osteopontin and increased collagen content in human ascending thoracic aortic aneurysms. *Vascular, 22*(1), 20-27. https://doi.org/10.1177/1708538112472282

Ponticos, M. (2013). Connective tissue growth factor (CCN2) in blood vessels. *Vascular Pharmacology, 58*(3), 189-193. https://doi.org/10.1016/j.vph.2013.01.004

Quan, T., Shao, Y., He, T, Voorhees, J.J., & Fisher, G.J. (2010). Reduced expression of connective tissue growth factor (CTGF/CCN2) mediates collagen loss in chronologically aged human skin. *The Journal of investigative dermatology, 130*(2), 415–424. https://doi.org/10.1038/jid.2009.224

Sachdeva, J., Mahajan, A., & Cheng, J., Baeten, J.T., Lilly, B., Kuivaniemi, H., & Hans, C.P. (2017). Smooth muscle cell-specific Notch1 haploinsufficiency restricts the progression of abdominal aortic aneurysm by modulating CTGF expression. *PLoS ONE, 12*(5), e0178538. https://doi.org/10.1371/journal.pone.0178538

Ungvari, Z., Valcarcel-Ares, M.N., Tarantini, S., Yabluchanskiy, A., Fulop, G.A., Kiss, T., & Csiszar, A. (2017). Connective tissue growth factor (CTGF) in age-related vascular pathologies. *GeroScience, 39*(5-6), 491–498. https://doi.org/10.1007/s11357-017-9995-5