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Molecular mechanisms of left atrial fibrosis development in patients with atrial fibrillation and metabolic syndrome: what biomarkers should be used in clinical practice?

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Aim. To determine the blood concentration of fibrosis biomarkers in patients with atrial fibrillation (AF) in combination with metabolic syndrome (MS) and to analyze the relationship with myocardial fibrosis.

Material and methods. This cross-sectional case-control study included 547 patients aged 35 to 65 years: experimental group — patients with MS (n=373), of which 202 patients had AF; comparison group — AF patients without MS (n=110); healthy subjects without cardiovascular diseases and metabolic disorders (n=64). Patients with AF and MS who underwent electroanatomic mapping before pulmonary vein isolation (n=79) were assessed for left atrial (LA) fibrosis severity.

Results. It was found that the blood concentration of circulating profibrogenic biomarkers in patients with AF and MS is higher than in patients with AF without MS: aldosterone (135,1 (80,7-224,1) and 90,1 (68,3-120,3) pg/ml, p<0,0001), galectin-3 (10,6 (4,8-15,4) and 5,8 (4,8-8,3) pg/ml, p=0,0001), GDF15 (938,3 (678,3-1352,1) and 671,0 (515,7-879,5) pg/ml, p=0,001), TGF-beta-1 (4421,1 (2513,5-7634,5) and 2630,5 (2020,7-3785,4) pg/ml, p=0,001), CTGF (167,8 (78,9-194,3) and 124,3 (74 , 4-181,9) pg/ml, p<0,0001), PIIINP (88,5 (58,6-120,4) and 58,9 58,9 (40,7-86,1) ng/ml, p<0,0001), PINP (3421,4 (1808,1-4321,7) and 2996,1 (2283,8-3894,3) pg/ml, p<0,0001). Patients with paroxysmal AF have higher concentrations of TGF-beta1, CTGF and PINP than patients with persistent and permanent AF. In patients with persistent AF and MS, the concentrations of galectin-3, aldosterone, and PIIINP were higher than in patients with paroxysmal AF, while in patients with permanent AF, they were significantly lower. The plasma concentration of galectin-3 positively correlated with levels of PINP (ρ=0,465, p<0,0001), PIIINP (ρ=0,409, p<0,0001), GDF-15 (ρ=0,369, p<0,0001), CTGF (ρ=0,405, p<0,0001). According to multivariate regression, of all studied biomarkers, GDF-15 had a greater effect on PIIINP concentration (β=0,234, p=0,038), and galectin-3 — on PINP (β=0,248, p<0,021). Positive correlations of the severity of left atrial fibrosis with the concentration of galectin-3 (ρ=0,563, p<0,0001), PINP (ρ=0,620, p<0,0001), TGF-beta-1 (ρ=0,390, p<0,0001) and CTGF (ρ=0,551, p<0,0001). According to linear multivariate regression, the most significant effect on LA fibrosis severity among the studied biomarkers is exerted by galectin-3 (β=0,432, p<0,0001), PINP (β=0,343, p=0,001) and PIIINP (β=0,286, p=0,008).

Conclusion. An increase in the blood concentration of profibrogenic biomarkers galectin-3, TGF-beta-1, CTGF, PIIINP, and PINP is associated with an increase in LA fibrosis severity and probably has a pathogenetic role in increasing the AF risk in patients with MS.

Keywords: aldosterone, galectin-3, GDF-15, TGF-beta-1, CTGF, PINP, PIIINP, atrial fibrillation, metabolic syndrome.

Relationships and Activities: none.

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Prevalence of atrial fibrillation (AF) in developed countries is 1,5% [1]. Metabolic syndrome (MS) increases the risk of AF by 67%, as previously shown in the prospective Atherosclerosis Risk in Communities Study (ARIC). Among all the MS components, cardiac remodeling is primarily affected by hypertension (HTN) and abdominal obesity [2, 3]. HTN promotes AF due to increased afterload and developing cardiac remodeling, as well as due to hyperactivation of renin-angiotensin-aldosterone system (RAAS), which leads to myocardial structural changes with the formation of arrhythmia substrate — left atrial (LA) fibrosis [3]. Evidence for the role of HTN and RAAS in AF development is the fact that primary aldosteronism, characterized by HTN and high blood aldosterone levels, increases the risk of AF by 12 times [4]. Recently, special attention has been paid to obesity, primarily visceral obesity, as a possible cause of AF [5]. Obesity causes hemodynamic impairment — an increase in circulating plasma volume, contributing to dilatation of cardiac cavities, including LA. In addition, adipose tissue has a powerful fibrogenic and pro-inflammatory effect on cardiovascular system, promotes apoptosis and fibrosis. It is known that the development of AF is based on electrical instability of myocardium, its remodeling and structural remodeling. That is why such profibrotic conditions as HTN, heart failure, myocardial infarction, inflammation, obesity, and diabetes play a key role in the predisposition to arrhythmias. At the same time, the molecular mechanisms underlying the remodeling of atrial extracellular matrix are still not fully defined. Currently, a number of humoral factors have been established that have a fibrogenic effect: angiotensin II, aldosterone, galectin-3, transforming growth factor-beta1 (TGF-beta1), platelet- derived growth factor (PDGF) and connective tissue growth factor (CTGF), the role of which in AF development is currently being actively studied [6-9]. Studies on the problem of fibrogenesis previously showed that aldosterone regulates the production of TGF-beta1 and CTGF, which, in turn, are pleiotropic triggers for fibroblast activation, production of types I and III procollagen, and development of fibrosis [10, 11]. Understanding the mechanisms of AF development, including in MS, is essential for creation of approaches to its prevention and treatment. The search for myocardial fibrosis markers and predictors of AF development and progression in patients with MS is extremely important, because this will make it possible to identify groups at risk of developing AF and to provide primary and secondary prevention.

The aim of study was to determine the association of blood plasma levels of fibrosis markers (aldosterone, galectin-3, growth differentiation factor-15 (GDF-15), TGF-beta1, CTGF, N-terminal propeptide of procollagen type I and III) with the severity of left atrial fibrillation fibrosis in patients with AF and MS, as well as to identify AF predictors in patients with MS.

Material and methods

In the period from 2014 to 2018, 1307 patients with AF admitted to therapy department of the University Clinic were examined, of which 721/1307 (55,2%) patients were diagnosed with coronary artery disease (CAD), 46/1307 (3,5%) — valvular heart disease, 80/1307 (6,1%) — inflammatory heart diseases. Further prospective follow-up included 547 subjects of both sexes aged 35-65 years: those with AF and MS (n=202); with AF and without MS (n=110); with MS without AF (n=171); healthy subjects (n=64). First and second groups included patients with paroxysmal (n=193), persistent (n=70) and permanent (n=49) AF types.

The study was carried out in accordance with the Good Clinical Practice (GCP) and the Declaration of Helsinki standards. The study protocol was approved by the Ethics Committee of the First Pavlov State Medical University of St. Petersburg. All patients signed written informed consent.

All MS patients had 3 or more components diagnosed according to the IDF criteria (2005). The study excluded patients with acute diseases and exacerbations of chronic inflammatory diseases, valvular heart disease, systemic diseases and cancer, as well as patients with impaired renal and liver function, thyroid diseases and primary hyperaldosteronism, strokes, cardiac surgery or other interventions in history. In all participants, anthropometric and diagnostic investigations were assessed, including electrocardiography and echocardiography. Echocardiography was performed using a Vivid 7 ultrasound system (GE, USA).

All samples of plasma and serum were centrifuged, followed by freezing at -40° C and determination of concentration of studied biomarkers using standard kits of enzyme-linked immunosorbent assay (ELISA). Aldosterone level was determined in blood plasma using an ELISA kit from DBC Inc (Canada). The level of galectin-3 in blood serum was determined by ELISA kit from eBioscience (Austria) with a detection range of 0,47-30,0 ng/ml. GDF-15 was determined in plasma by ELISA kit using BioVendor Human GDF-15/MIC-1 reagent kit (Czech Republic); the minimum detection value was 16,0 pg/ml. The concentration of TGF-beta1 was determined in blood serum by ELISA kit using the ProcartaPlex Human TGF-beta1 Simplex reagent kit from Affymetrix (eBioscience)

Table 1

Clinical, laboratory and echocardiographic characteristics of patients

Parameters		MS- AF- n=64 (1)	MS+ AF- n=171 (2)	MS- AF+ n=110 (3)	MS+ AF+ n=202 (4)	Statistical significance, p
Age, years		54,3±7,6	57,7±8,2	56,2±6,8	58,1±5,2	p>0,05
Sex, male/female		30/33	68/51	33/50	80/69	p>0,05
BMI, kg/m^2		21,5±3,8	32,3±7,3	24,2±5,1	31,1±7,1	$p_{1,2}$<0,001 $p_{1,4}$<0,001
Waist circumference, cm		76,5±5,1	112,3±10,5	79,1±10,7	113,9±13,5	$p_{1,2}$<0,001 $p_{1,4}$<0,001
Total cholesterol, mmol/l		4,9±0,9	5,4±1,1	4,8±1,2	5,2±1,2	$p_{1,2}$<0,001 $p_{1,4}$<0,001
LDL-C, mmol/l		2,8±0,3	3,4±0,3	3,1±0,3	3,1±0,4	$p_{1,2}$<0,001 $p_{1,3}$<0,001 $p_{1,4}$<0,001
HDL-C, mmol/l		1,6±0,3	1,2±0,3	1,4±0,3	1,3±0,4	$p_{1,2}$<0,001 $p_{1,4}$<0,001
TG, mmol/l		1,0±0,3	2,1±0,8	1,3±0,4	1,7±1,2	$p_{1,2}$<0,001 $p_{1,4}$<0,001
Glucose, mmol/l		4,7±0,6	6,1±1,2	5,1±0,4	6,0±1,4	$p_{1,2}$<0,001 $p_{1,4}$<0,001
Echocardiography						
LA diameter, mm		34,9±2,7	44,6±4,2	43,2±2,0	44,5±4,0	$p_{1,2}$<0,001 $p_{1,3}$<0,001 $p_{1,4}$<0,001
LA volume, ml		43,2±9,4	81,9±16,6	60,4±19,8	79,9±19,4	$p_{1,2}$<0,001 $p_{1,3}$<0,001 $p_{1,4}$<0,001 $p_{2,3}$=0,01 $p_{3,4}$=0,01
LA volume index, ml/m^2		24,3±4,9	39,2±9,7	30,4±9,0	40,1±11,2	$p_{1,2}$<0,001 $p_{1,3}$<0,001 $p_{1,4}$<0,001 $p_{2,3}$=0,01 $p_{3,4}$=0,01
LA volume, ml		41,3±8,9	68,5±14,4	57,5±20,6	65,9±14,7	$p_{1,2}$<0,001 $p_{1,3}$<0,001 $p_{1,4}$<0,001 $p_{2,3}$=0,01 $p_{3,4}$=0,01
RA volume index, ml/m^2		23,4±4,3	31,9±7,3	29,2±8,8	32,8±7,8	$p_{1,2}$<0,001 $p_{1,3}$<0,001 $p_{1,4}$<0,001
LVEF, %		65,3±7,0	63,2±6,0	62,4±4,2	61,4±6,0	p>0,05
AF duration, years		-	-	4,4±1,2	4,6±2,2	p>0,05
AF type	Paroxysmal	-	-	83/110 (75,4%)	110/202 (54,4%)	
	Persistent	-	-	20/110 (18,2%)	50/202 (24,8%)	
	Permanent	-	-	7/110 (6,4%)	42/202 (20,8%)	

Abbreviations: BMI — body mass index, LDL-C — low density lipoprotein cholesterol, HDL-C — high density lipoprotein cholesterol, MS — metabolic syndrome, TG — triglycerides, LA — left atrium, RA — right atrium, LVEF — left ventricular ejection fraction, AF — atrial fibrillation.

(Austria); the minimum detection value was 8,6 pg/ml. The concentration of CTGF was determined in blood plasma by ELISA kit using Human CTGF (High Sensitive) Aviscera Bioscience Inc. reagent kit; the minimum detection value was 30,0 pg/ml. Concentrations procollagen type I N-terminal propeptide (PINP) and procollagen type III N-terminal propeptide (PIIINP) were

Table 2

Plasma and serum concentrations of aldosterone, galectin-3, GDF-15, TGF-beta1, CTGF, PIIINP and PINP in patients with AF and MS

Biomarkers	MS- AF- n=64 (1)	MS+ AF- n=171 (2)	MS- AF+ n=110 (3)	MS+ AF+ n=202 (4)	Statistical significance, p
Aldosterone, pg/ml	97,0 (55,8-125,5)	122,5 (87,0-173,5)	90,1 (68,3-120,3)	135,1 (80,7-224,1)	$p_{1,2}<0,0001$ $p_{1,3}=0,625$ $p_{1,4}<0,0001$ $p_{2,3}=0,01$ $p_{2,4}=0,01$ $p_{3,4}<0,0001$
Galectin-3, ng/ml	3,2 (2,4-4,2)	4,9 (4,2-8,8)	5,8 (4,8-8,3)	10,6 (4,8-15,4)	$p_{1,2}=0,001$ $p_{1,3}<0,0001$ $p_{1,4}<0,0001$ $p_{2,3}=0,467$ $p_{2,4}<0,0001$ $p_{3,4}=0,0001$
GDF-15, pg/ml	439,1 (410,2-474,6)	739,7 (541,9-996,7)	671,0 (515,7-879,5)	938,3 (678,3-1352,1)	$p_{1,2}<0,0001$ $p_{1,3}<0,0001$ $p_{1,4}<0,0001$ $p_{2,3}=0,446$ $p_{2,4}=0,001$ $p_{3,4}=0,001$
TGF-beta1, pg/ml	1840,5 (1414,3-3720,4)	2560,4 (2145,3-4515,8)	2630,5 (2020,7-3785,4)	4421,1 (2513,5-7634,5)	$p_{1,2}=0,01$ $p_{1,3}=0,002$ $p_{1,4}<0,0001$ $p_{2,3}=0,929$ $p_{2,4}=0,002$ $p_{3,4}=0,001$
CTGF, pg/ml	74,5 (45,5-103,8)	133,0 (91,3-172,5)	124,3 (74,4-181,9)	167,8 (78,9-194,3)	$p_{1,2}<0,0001$ $p_{1,3}=0,001$ $p_{1,4}<0,0001$ $p_{2,3}=0,347$ $p_{2,4}<0,0001$ $p_{3,4}<0,0001$
PIIINP, ng/ml	33,3 (23,5-42,6)	55,1 (37,7-86,9)	58,9 (40,7-86,1)	88,5 (58,6-120,4)	$p_{1,2}<0,0001$ $p_{1,3}<0,0001$ $p_{1,4}<0,0001$ $p_{2,3}=0,178$ $p_{2,4}<0,0001$ $p_{3,4}<0,0001$
PINP, pg/ml	1278,8 (775,1-2266,6)	2130,3 (1392,0-2820,1)	2996,1 (2283,8-3894,3)	3421,4 (1808,1-4321,7)	$p_{1,2}=0,008$ $p_{1,3}<0,0001$ $p_{1,4}<0,0001$ $p_{2,3}=0,067$ $p_{2,4}<0,0001$ $p_{3,4}=0,01$

Abbreviations: MS — metabolic syndrome, AF — atrial fibrillation, GDF-15 — growth differentiation factor 15, TGF-beta1 — transforming growth factor-beta1, CTGF — connective tissue growth factor, PINP — N-terminal propeptide of type I procollagen, PIIINP — N-terminal propeptide of type III procollagen.

determined in blood plasma by ELISA kit from Cloud-Clone Corp. (USA) with a detection range of 33-5000 pg/ml and 2,14-400 ng/ml, respectively.

In patients with ineffective antiarrhythmic therapy, indications for interventional treatment of AF have been determined. In an X-ray operation room using a CARTO3 non-fluoroscopic electroanatomical mapping system (Biosense Webster, USA) and a contact force sensing catheter (Smart Touch Thermocool, Biosense Webster, USA), with a sinus rhythm, bipolar amplitude maps of LA and local activation time maps was created. Evaluation of low-voltage areas of 0,2-1,0 mV using the Area Measurement tool was carried out in the off line mode. The prevalence of fibrosis was estimated as a percentage of total LA area.

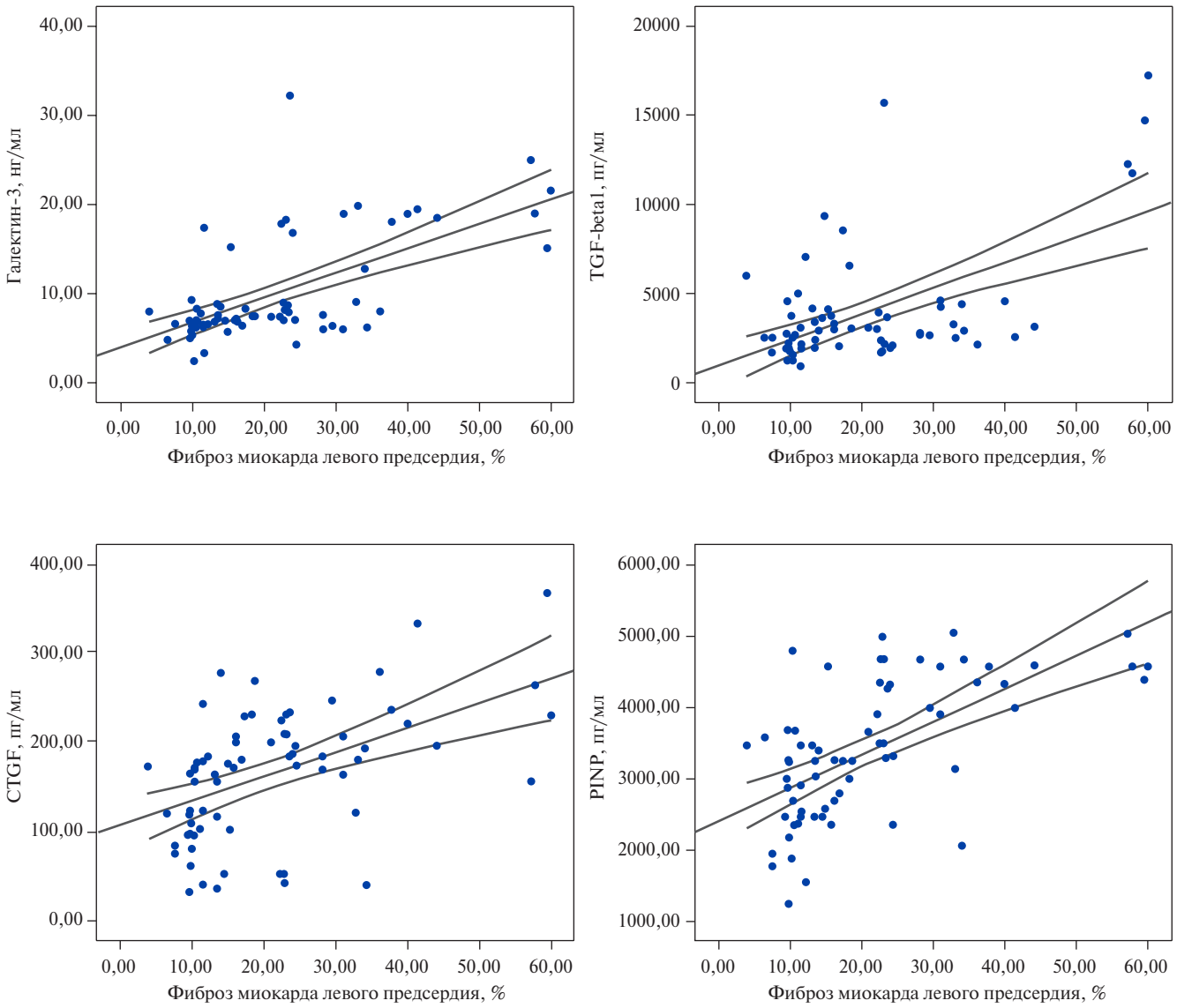

Figure 1. Correlations of galectin-3, TGF-beta1, CTGF and PINP with the severity of myocardial fibrosis in patients with AF and MS.
Abbreviations: TGF-beta1 — transforming growth factor-beta1, CTGF — connective tissue growth factor, PINP — N-terminal propeptide of type I procollagen.

The results were entered into the original database. Distribution normality of the numerical variables was assessed using the Kolmogorov-Smirnov test. Depending on distribution type, normally distributed quantitative variables are presented by the mean (M) ± standard deviation (σ). Parametric unpaired Student's t-test was used for comparison in independent groups of normally distributed variables. For nonnormally distributed quantitative variables, the data are presented as a median (Me) and interquartile intervals (25-75%). For comparison in independent groups, the nonparametric Mann-Whitney U-test was used. Multiple comparisons in groups (more than two) in parametric statistics were carried out using one-way variance analysis (ANOVA), and for nonparametric statistics — the Kruskal-Wallis test. In this case, the Bonferroni correction was used. When assessing the significance of correlation coefficient, we used the Pearson (r) tests with a normal distribution and Spearman (ρ) with a nonnormal distribution. We also used linear univariate and multivariate regression analyzes to assess the influence of factors on quantitative variables and binomial regression analysis to predict the probability of event. Statistical analysis was performed using licensed IBM SPSS software, version 22.0.

Results

The study groups were comparable in sex distribution and did not differ significantly by age. The main

Table 3

Plasma and serum concentrations of aldosterone, galectin-3, GDF-15, TGF-beta1, CTGF, PIIINP and PINP in patients with MS and various AF types

Biomarkers	Paroxysmal AF n=110 (1)	Persistent AF n=50 (2)	Permanent AF n=42 (3)	Statistical significance, p
Aldosterone, pg/ml	140,5 (90,4-182,2)	221,5 (107,4-272,9)	85,1 (72,5-111,5)	$p_{1,2}$=0,001 $p_{1,3}$<0,0001 $p_{2,3}$<0,0001
Galectin-3, ng/ml	8,9 (5,0-14,4)	12,5 (5,2-18,3)	6,3 (3,3-12,9)	$p_{1,2}$=0,001 $p_{1,3}$=0,001 $p_{2,3}$<0,0001
GDF-15, pg/ml	792,1 (619,9-1101,1)	982,4 (787,1-1252,0)	1345,0 (1102,7-1729,2)	$p_{1,2}$<0,0001 $p_{1,3}$<0,0001 $p_{2,3}$<0,0001
TGF-beta1, pg/ml	3678,5 (2279,8-6700,6)	2829,9 (1588,8-4349,4)	2228,3 (1839,9-2459,1)	$p_{1,2}$=0,015 $p_{1,3}$=0,001 $p_{2,3}$=0,651
CTGF, pg/ml	172,5 (106,7-208,5)	118,8 (79,2-200,4)	78,6 (61,6-97,5)	$p_{1,2}$<0,0001 $p_{1,3}$<0,0001 $p_{2,3}$=0,001
PIIINP, ng/ml	76,4 (55,6-120,5)	101,3 (84,9-147,1)	78,5 (62,3-89,7)	$p_{1,2}$<0,0001 $p_{1,3}$=0,417 $p_{2,3}$⩽0,0001
PINP, pg/ml	4567,2 (2456,9-5567,4)	2806,3 (1600,0-4234,1)	1893,6 (1470,3-2521,1)	$p_{1,2}$=0,018 $p_{1,3}$=0,001 $p_{2,3}$=0,003

Abbreviations: AF — atrial fibrillation, GDF-15 — growth differentiation factor 15, TGF-beta1 — transforming growth factor-beta1, CTGF — connective tissue growth factor, PINP — N-terminal propeptide of type I procollagen, PIIINP — N-terminal propeptide of type III procollagen.

clinical, laboratory and echocardiographic characteristics are presented in Table 1.

Table 2 presents data on serum and plasma concentrations of studied biomarkers in subjects. It was found that the blood concentrations of aldosterone and galectin-3 in patients with AF and MS are higher than in patients with MS without AF and higher than in healthy subjects. The highest concentration of TGF-beta1 was found in patients with AF and MS, and there were no significant differences in its concentration in the groups of patients with MS without AF and AF without MS. Serum TGF-beta1 levels in patients with MS and without AF and those with AF and without MS did not differ, but were higher than in healthy subjects. The serum concentration of CTGF in patients with AF and MS was higher than in patients with MS and without AF and higher than in healthy subjects.

As for fibrosis biomarkers, it was found that patients with persistent AF and MS had higher levels of aldosterone, galectin-3, and PIIINP than patients with paroxysmal and permanent AF. Plasma concentration of GDF-15 was higher in patients with permanent AF than in patients with paroxysmal and persistent AF. In patients with paroxysmal AF and MS, plasma levels of CTGF and PINP were higher than in patients with persistent and permanent AF. TGF-beta1 was higher in paroxysmal AF than in patients with persistent and permanent AF. The data are presented in Table 3.

It was found that the concentration of galectin-3 in blood plasma positively correlated with PINP levels (ρ=0,465, p<0,0001), PIIINP (ρ=0,409, p<0,0001), GDF-15 (ρ=0,369, p<0,0001), CTGF (ρ=0,405, p<0,0001). The concentration of GDF-15 correlated to a greater extent with PIIINP (ρ=0,403, p<0,0001) than with PINP (ρ=0,232, p=0,03). In turn, TGF-beta1 correlated more significantly with PIIINP (ρ=0,329, p<0,0001), and CTGF — with PINP (ρ=0,386, p<0,0001). According to multivariate regression analysis, of all studied biomarkers, GDF-15 had a greater effect on PIIINP concentration (β=0,234, p=0,038), and galectin-3, on PINP (β=0,248, p<0,021).

Analysis of voltage maps and assessing the severity of LA myocardial fibrosis in AF patients who underwent pulmonary vein isolation revealed that LA fibrosis is more common in patients with AF and MS than in AF patients without MS (26,1 (14,5-41,5)% and 10,5 (7,3-16,2)%, p=0,028). Assessment of the relationship of circulating biomarkers in patients with AF and MS, who underwent electroanatomic

Table 4

Blood concentrations of fibrosis biomarkers in patients with AF and MS with varying severity of LA fibrosis

% of LA fibrosis, quartiles (Q)	⩽12,3% (Q1)	12,4-22,4% (Q2)	22,5-33,4% (Q3)	⩾33,5% (Q4)	Statistical significance, p
Aldosterone, pg/ml	8,6 (5,9-13,4)	7,4 (6,7-16,0)	8,8 (6,4-18,3)	12,34 (9,3-18,8)	$p_{1,2}$=0,06 $p_{1,3}$=0,647 $p_{1,4}$=0,001 $p_{2,3}$=0,08 $p_{2,4}$=0,001 $p_{3,4}$=0,001
Galectin-3, ng/ml	68,7 (66,1-86,3)	110,0 (75,6-117,1)	89,0 (80,0-120,0)	123,4 (92,6-198,8)	$p_{1,2}$=0,001 $p_{1,3}$=0,01 $p_{1,4}$<0,0001 $p_{2,3}$=0,07 $p_{2,4}$<0,0001 $p_{3,4}$=0,0001
GDF-15, pg/ml	725,9 (613,5-854,1)	641,5 (534,6-829,1)	687,5 (554,8-1501,4)	1020,1 (669,1-1243,9)	$p_{1,2}$=0,05 $p_{1,3}$=0,124 $p_{1,4}$<0,0001 $p_{2,3}$=0,236 $p_{2,4}$<0,0001 $p_{3,4}$<0,0001
TGF-beta1, pg/ml	1961,6 (1434,9-1327,4)	3934,6 (3203,9-5355,4)	2643,1 (2165,9-4259,4)	3678,2 (2348,9-4751,4)	$p_{1,2}$=0,01 $p_{1,3}$=0,04 $p_{1,4}$<0,0001 $p_{2,3}$=0,929 $p_{2,4}$=0,245 $p_{3,4}$=0,147
CTGF, pg/ml	179,8 (164,5-224,6)	175,4 (128,4-203,4)	120,9 (42,8-210,3)	220,9 (178,9-306,4)	$p_{1,2}$=0,359 $p_{1,3}$=0,01 $p_{1,4}$<0,0001 $p_{2,3}$=0,04 $p_{2,4}$<0,0001 $p_{3,4}$<0,0001
PIIINP, ng/ml	58,3 (49,8-90,9)	59,4 (47,7-63,4)	61,6 (57,4-105,5)	92,2 (66,4-125,1)	$p_{1,2}$=0,959 $p_{1,3}$=0,781 $p_{1,4}$<0,0001 $p_{2,3}$=0,854 $p_{2,4}$<0,0001 $p_{3,4}$<0,0001
PINP, pg/ml	2839,1 (1412,1-3458,1)	2986,1 (2623,1-3571,1)	3567,1 (2498,1-4986,1)	4344,1 (4122,1-4567,1)	$p_{1,2}$=0,899 $p_{1,3}$=0,01 $p_{1,4}$<0,0001 $p_{2,3}$=0,04 $p_{2,4}$<0,0001 $p_{3,4}$<0,0001

Abbreviations: LA — left atrium, GDF-15 — growth differentiation factor 15, TGF-beta1 — transforming growth factor-beta1, CTGF — connective tissue growth factor, PINP — N-terminal propeptide of type I procollagen, PIIINP — N-terminal propeptide of type III procollagen.

mapping before pulmonary vein isolation (n=79), revealed positive correlations of LA myocardial fibrosis severity with the concentration of galectin-3 (ρ=0,563, p<0,0001), PINP (ρ=0,620, p<0,0001), TGF-beta1 (ρ=0,390, p<0,0001) and CTGF (ρ=0,551, p<0,0001), which shown in Figure 1. For detailed analysis, these AF and MS patients were divided into groups depending on the proportion of LA fibrosis, divided into quartiles (25%, 50%, 75%). It was found that patients with the highest severity of LA fibrosis (⩾33% — Q4) had higher concentrations of galectin-3, aldosterone, GDF-15, PINP, and PIIINP (Table 4). Linear multivariate regression established that the most significant effect on LA fibrosis severity (% of fibrosis) was exerted by the following biomarkers: galectin-3 (β=0,432, p<0,0001), PINP (β=0,343, p=0,001), and PIIINP (β=0,286, p=0,008).

Discussion

AF pathogenesis is a comprehensive process, which is based on hemodynamic, structural, electrophysiological and molecular mechanisms. There are many clinical risk factors for AF, including advanced age, CAD, thyroid diseases, heart failure, chronic pulmonary diseases, etc. [1]. Our study did not include patients with organic heart diseases, acute and chronic significant comorbidities. In patients with MS, in contrast to the comparison groups, 3 or more MS components were diagnosed, the most frequent among which were HTN, abdominal obesity, and dyslipidemia. HTN contributes to left ventricular hypertrophy and diastolic dysfunction, as well as LA dilatation. The mechanisms of myocardial structural remodeling, including atria, aldosterone plays an important role. The profibrotic role of aldosterone is known and is currently not in doubt. Through the development of fibrosis, which is a possible substrate of nonvalvular AF, aldosterone, in turn, can act as a predictor of this arrhythmia. In 2015, the first data on the role of galectin-3 and aldosterone in AF development were published and it was found that the concentrations of these biomarkers are higher in patients with AF in combination with MS, compared with patients with MS but without AF [12]. This study found that the concentration of aldosterone and galectin-3 in patients with AF combined with MS is higher than in patients with isolated AF without MS, which, in turn, emphasizes the relationship of these biomarkers with MS and its contribution to AF. It is known from experimental studies that aldosterone acts as one of the main initial inducers of cascade of profibrotic factors. It was found that aldosterone induces the secretion of galectin-3 through hyperactivation of macrophages, which, in turn, induces the production of TGF-beta1, while CTGF — effector molecules involved in the activation of fibroblasts and excessive production of PINP and PIIINP into the extracellular matrix [6, 13]. An experimental work by Schreier B. et al. found that aldosterone is able to induce fibrosis in the myocardium and kidneys through the activation of TGF-beta1 synthesis and, as a consequence, an increase in procollagen production, followed by developing heart failure [14]. Aldosterone induces TGF-beta1 synthesis, but at the same time, TGF-beta1 mutually enhances the production of aldosterone and other profibrogenic factors through a positive feedback [15]. TGF-beta1 is a member of a protein family that play a critical role in epithelial-to-mesenchymal transition during embryogenesis of valvular and septal cardiac structures and is secreted by various cells, such as cardiomyocytes, fibroblasts, endothelial cells and inflammatory effectors. Various causes underlying the onset of AF increase TGF-beta1 expression level, which, in turn, induces interstitial fibrosis [16]. In addition, TGF-beta1 is able to regulate transcription and function of cardiomyocyte sodium channels [17].

We found that plasma concentration of TGF-beta1 in patients with AF and MS is higher than in patients with isolated AF and is significantly higher than in healthy subjects. While TGF-beta1 is a key inducer of fibrosis, another known growth factor (CTGF) promotes it [18]. CTGF is a member of CCN protein family (Cyr61, CTGF, nov), which is one of the main downstream effectors in the development of TGF-beta1-induced fibrosis. In myocardial with active remodeling, combined expression of CTGF and TGF-beta1 was revealed [19]. Ko WC, et al. found that gene expression and secretion of CTGF in atrial tissue of patients with CAD and AF is higher than in patients with sinus rhythm. The authors also found that in an experimental animal model, the administration of angiotensin II caused an increase in CTGF concentration and fibroblast proliferation with the formation of type I collagen, which undoubtedly contributed to AF onset [9]. Lavall D, et al., during the cultivation of atrial tissue obtained from atrial biopsy in patients with mitral valve disease and AF, revealed that aldosterone increased CTGF secretion with activation of fibroblasts and an increase in extracellular matrix mass [20]. CTGF activity is regulated by angiotensin II and TGF-beta1, which enhances the development of fibrosis [21]. In a cohort of patients with AF and MS, we found that the plasma concentration of CTGF is significantly higher than in patients with isolated AF and isolated MS. A positive correlation was found with galectin-3 and circulating procollagens. In the pathogenesis of fibrosis development, galectin-3 is a key factor that triggers complex myocardial remodeling processes. On the one hand, it is able to influence matrix metalloproteinases, limiting extracellular matrix degradation. On the other hand, it activates fibroblasts and increases the synthesis of collagen types I and III, which has been well studied in experimental animal models [22]. PINP and PIIINP are deposited directly in the myocardium in various heart diseases. In particular, a study by Lopez B, et al. established an association between circulating PINP and development of LV myocardial remodeling in patients with HTN [23]. The relationship of these biomarkers with the risk of AF has been established in numerous studies earlier; moreover, it has been proven that in patients with AF, the deposition of type I procollagen, rather than type III procollagen, predominates in LA tissue [24, 25].

According to our study, it was found that plasma concentration of PINP and PIIINP is higher in patients with AF and MS in comparison with patients with isolated AF MS and higher than in patients with isolated MS. A positive correlation of galectin-3, TGF-beta1, and CTGF with plasma concentrations of circulating PINP and PIIINP was established, which emphasizes the relationship between these biomarkers in the development of fibrosis.

Additional analysis of data from patients with AF in combination with MS on various AF types revealed that patients with the paroxysmal AF have higher concentrations of TGF-beta1, CTGF, and PINP than patients with persistent and permanent AF. Probably, these biomarkers play a more significant role in the development of AF in patients with MS in early stages of arrhythmia. On the other hand, in patients with persistent AF and MS, the blood concentrations of galectin-3, aldosterone, and PIIINP were higher than in patients with paroxysmal AF, and in patients with permanent AF, they were significantly lower, which suggests that that these biomarkers to a greater extent specifies the progression of arrhythmia. Attention is also drawn to the fact that the concentration of GDF-15 is highest in patients with permanent AF. This biomarker is also important in pathogenesis of arrhythmia and is a predictor of unfavorable prognosis regarding the risk of cardiovascular events in patients with AF [26]. The study found that in patients with AF in combination with MS, the concentration of GDF-15 is higher than in patients with isolated AF, which is probably due to MS components, since the production of this biomarker is enhanced by inflammation, hypoxemia, and metabolic disorders [27].

Analysis of the data on severity of LA myocardial fibrosis in AF patients who underwent radiofrequency pulmonary vein isolation established that the prevalence of LA fibrosis in patients with AF in combination with MS is higher than in isolated AF. Previously, we published data that the severity of LA myocardial fibrosis in patients with AF and MS is greater than in patients with AF without MS, and is associated with galectin-3 [28]. Currently, more data have been obtained on the relationship of biomarkers with LA myocardial fibrosis. Positive correlations of the severity of LA myocardial fibrosis were revealed not only with galectin-3 and TGF-beta1, but also with PINP and CTGF, which was confirmed by linear univariate regression. Linear multivariate regression revealed that the prevalence of LA myocardial fibrosis was more significantly influenced by the following biomarkers: galectin-3, PIIINP and PINP. The analysis of concentrations of fibrosis biomarkers in patients with AF in combination with MS in subgroups of patients with different severity of LA fibrosis made it possible to establish that the studied biomarkers are significantly increased in patients with the most severe fibrosis (>33,5%).

Thus, this study investigated the role of molecular fibrosis markers in the pathogenesis of AF in patients with MS. Determination of markers of myocardial fibrosis and predictors of development and progression of AF in patients with MS is of great importance, because will allow identifying patients at high risk of developing AF and carrying out its primary and secondary prevention. Fibrogenic factors may act as a potential target for therapy in patients with AF in combination with MS.

Study limitations. Patients with AF and MS received medications (antiarrhythmic, antihypertensive, antithrombotic, statins), which could to some extent affect the study results. The number of patients assessed for severity of LA fibrosis was relatively small. Therefore, it is necessary to collect more data and conduct further monitoring of patients with MS. To study the prognostic role of studied biomarkers, prospective follow-up of patients should be continued to obtain data on potential of their use in clinical practice.

Conclusion

1. The blood concentration of aldosterone, galectin-3, TGF-beta1, GDF-15, CTGF, PIIINP and PINP in patients with AF in combination with MS is higher than in patients with isolated AF and isolated MS.

2. In patients with paroxysmal AF and MS, the levels of TGF-beta1, CTGF and PINP are higher than in patients with persistent and permanent AF. In patients with persistent AF and MS, the concentrations of galectin-3, aldosterone and PIIINP is higher than in patients with paroxysmal AF.

3. The concentration of galectin-3, TGF-beta1, CTGF, PIIINP and PINP are associated with a severe LA myocardial fibrosis in patients with AF in combination with MS.

Relationships and Activities: none.

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