The soluble ST2 level predicts risk of atrial fibrillation recurrences in long-term period after radiofrequency ablation

Tatiana P. Gizatulina[1*], Aleksandra V. Mamarina[1], Leysan U. Martyanova[1], Denis V. Belonogov[1], Grigoriy V. Kolunin[1], Tatiana I. Petelina[1], Nikita E. Shirokov[1] and Elena A. Gorbatenko[1]

Abstract

Background and objectives :The hypothesis of the study was the assumption that the serum levels of soluble ST2 (sST2) and growth differentiation factor (GDF-15) can be predictors of atrial fibrillation (AF) recurrence in long-term period after primary radiofrequency catheter ablation (RFA).

Methods Of the 165 patients included in the prospective follow-up, the final analysis included 131 patients whose follow-up duration reached 18 months after the end of the blanking period (3 months after RFA). The median age of patients was 59.0 (50.0; 64.0) years, and 80 (61%) were men. Paroxysmal AF was present in 103 (79%) and persistent AF in 28 (21%) patients. All patients underwent transthoracic and transesophageal echocardiography, and electroanatomic mapping was used to assess the area of low-voltage zones (LVZ). sST2 and GDF-15 levels were determined by ELISA using GDF-15/MIC-1 analytical kits (BioVender, Czech Republic) and Presage ST2 (Critical Diagnostics, USA) before RFA. After RFA, patients had regular follow-up visits at 3-6-9-12-18 months with 12-lead ECG or Holter ECG monitoring and with clinical evaluation. The primary endpoint was the occurrence of the first symptomatic AF recurrence (AFr) lasting > 30 s, recorded on an ECG or during daily ECG monitoring, after a blanking period.

Results At the 18-month follow-up, 47 patients (35.9%) had AFr. The groups with and without AFr didn`t differ in the LVZ area. The medians of NT-proBNP, GDF-15 and sST2 also didn`t differ significantly between the groups, but in patients with AFr, the proportion of those with sST2 ≥ 36 ng/ml (the border of the lower and middle terziles) was higher ($p = 0.03$). According to the one-factor Cox regression analysis, AFr were associated with four factors: AF history ≥ 1 year, early AFr (during the blanking period), left atrial appendage flow velocity (LAAFV) < 54 cm/sec and sST2 ≥ 36 ng/ml. In the multivariate Cox analysis two independent predictors of AFr were obtained: sST2 ≥ 36 ng/ml (HR = 3.8; 95% CI 1.5–9.8, $p = 0.006$) and LAAFV < 54 cm/sec (HR = 1.96; 95% CI 1.01–3.82, $p = 0.048$).

Conclusions Serum sST2 level with a cut-off value of 36 ng/ml or more can be used as a predictor of AF recurrence in the long-term period after primary RFA.

Keywords Atrial fibrillation, Left atrial fibrosis, Radiofrequency ablation, Biomarkers, sST2

*Correspondence:
Tatiana P. Gizatulina
gizatulinatp@infarkta.net

[1]Tyumen Cardiology Research Center, Tomsk National Research Medical Center of the Russian Academy of Sciences, 111 Melnikaite Street, Tyumen 625026, Russian Federation

Introduction

Atrial fibrillation (AF) is the most common arrhythmia and is associated with a five-fold increase in the risk of stroke and a two-fold increase in the risk of death [1]. Radiofrequency ablation (RFA), aimed at eliminating AF triggers by isolating the mouths of the pulmonary veins (PV), is used in the treatment of patients with AF as part of the rhythm control strategy. RFA is superior to drug therapy in maintaining sinus rhythm and improving quality of life; however, the effectiveness of this method is still insufficient: the frequency of AF recurrences within the first year after RFA is between 25% and 40% [2]. Markers to predict recurrence could help with selecting patients who would benefit the most from the procedure. This dictates the need to develop a method for predicting AF recurrence in order to personalize the selection of patients for RFA.

It is generally believed that AF recurrence may be related to the reconnection of PVs and the progression of the underlying arrhythmic substrate [2]. Moreover, the reconnection of PVs is usually the cause of early AF recurrences (within the first 3 months after RFA), while it is logical to assume that the progression of the arrhythmic substrate eventually leads to later onset AF recurrences [3]. Atrial fibrosis is the main pathological basis for the pathogenesis and progression of AF. The role of fibrosis biomarkers in AF has been widely discussed over the last decade. Of particular interest are data on a new circulating biomarker expressed by cardiomyocytes, fibroblasts, and endothelial cells, a member of the interleukin-1 (IL-1) receptor family, the soluble suppression of tumorigenicity 2 (sST2), whose ligand is the cytokine IL-33 [4]. It has been established that sST2 is a marker of myocardial fibrosis and structural remodeling of the heart [5], and its level reflects the risk of occurrence [6] and the progression of AF [4].

We have previously reported that the severity of the arrhythmogenic substrate, estimated by the area of low-voltage zones (LVZ) in the left atrium (LA) during electroanatomic mapping (EAM), is associated with an increase in the blood levels of growth differentiation factor 15 (GDF-15) [7, 8]. The hypothesis of this study was our assumption that determining the levels of circulating biomarkers sST2 and GDF-15 may help in predicting late AF recurrence after primary radiofrequency circular PV isolation. Currently, only a few studies have been devoted to studying the role of GDF-15 and sST2 in predicting AF recurrence after RFA [3]. At the same time, on one hand, data on the prognostic significance of these biomarkers in predicting AF recurrences after catheter ablation are contradictory [9], while, on the other, the cut-off values of these biomarkers predicting a high probability of AF recurrence also differ significantly. This confirms the relevance of this study.

Methods

This study was an open, single-center prospective study.

Inclusion criteria patients with symptomatic AF aged 18 to 75 years, men and women, the presence of indications for RFA [1, 2].

Exclusion criteria age<18 or >75 years, the presence of a mechanical prosthetic heart valve or moderate to severe mitral stenosis, thrombosis of left atrial appendage (LAA) detected by transesophageal echocardiography (TEE), myocardial infarction or coronary artery surgery within the last 6 months, left ventricular ejection fraction (LVEF)≤50%, acute or decompensation of chronic comorbidities, chronic obstructive pulmonary disease, pregnancy, refusal of the patients to participate in the study.

This study adhered to the guidelines outlined in the revised 2013 Helsinki Declaration, and informed consent was obtained from all patients individually. This study is supported by the Ministry of Education and Science of the Russian Federation (Project No. AAAA-A18-118041890067-9). The study protocol received approval from the Scientific Ethics Committee of the Tyumen Cardiology Research Center (protocol code 153 and 02/10/2019).

The study population included 165 patients with symptomatic AF referred for primary RFA in our center from January 2019 to August 2020. Patients who underwent RFA were included in a prospective observational study and had regular face-to-face or telephone follow-up visits with a doctor at 3, 6, 9, 12, and 18 month intervals, during which 12-lead electrocardiogram (ECG) or Holter ECG monitoring were recorded and a clinical evaluation was performed.

Primary endpoint the occurrence of the first symptomatic AF recurrence lasting>30 s recorded on an ECG or during daily ECG monitoring after a blanking period (the first 3 months after the date of RFA).

AF and comorbid diseases were diagnosed according to recommended guidelines. Paroxysmal AF was defined as self-terminating within 7 days of onset [1]. Persistent AF lasted longer than 7 days or required medication or electrical cardioversion for termination. All patients initially (before RFA) underwent general clinical examination, TEE and transthoracic echocardiography (TTE), and blood sampling for biomarkers (i.e., GDF-15, sST2, and N-terminal pro-brain natriuretic peptide [NT-proBNP]).

Echocardiographic assessment

TTE and TEE were performed within 48 h before RFA using a Vivid E9 ultrasound scanner (GE Medical Systems, USA) in accordance with the recommendations of

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the American Society of Echocardiography and the European Association for Cardiovascular Imaging [10, 11], where chamber size and volume as well as systolic and diastolic LV function were assessed.

TEE examinations were performed by qualified cardiac sonographers using a transesophageal matrix multiplane phased transducer. LAA scanning was performed from the middle esophageal view between 0° and 110° in 10–20° increments. To measure the left atrial appendage flow velocity (LAAFV) using a pulse-wave Doppler, blood samples were positioned 1 cm inside the entrance of the LAA. Subsequently, the average value of LAAFV was determined from 5 consecutive cardiac cycles.

Blood sampling and measurement of biomarkers

In addition to routine clinical laboratory tests and NT-proBNP (reference value<125 pg/mL), sST2 and GDF-15 levels were determined in all patients. Venous blood samples for the determination of GDF-15 and sST2 were collected the day before RFA after overnight fasting (8 h) and a 30 min rest in the sitting position. The samples were immediately centrifuged and stored at −80 °C until analysis. Later, the frozen serum samples were rapidly dissolved for analysis, which was performed 1 to 3 months after blood collection. GDF-15 and sST2 levels were determined using an enzyme immunoassay. GDF-15 in blood serum was determined using a human GDF-15/MIC-1 ELISA analytical kit (BioVender, Czech Republic). According to the instructions for the research kit, the medians of GDF-15 in different age groups can be taken as reference levels: 378–648 pg/mL for men and 444–653 pg/mL for women. sST2 levels were determined using "Critical diagnostic" kits (USA). The reference values for women and men are 7.1–33.5 ng/mL (average value 16.2 ng/mL) and 8.5–49.3 ng/mL (average value 23.6 ng/mL), respectively.

EAM and RFA

EAM was performed at a sinus rhythm before RFA. In patients with persistent AF, electrical cardioversion was performed beforehand. A 3D navigation system (Biosense Webster) was used for EAM. Thermocool Smart Touch ablation electrodes with an interelectrode distance of 3.5 mm and Lasso NAV multipolar circular mapping electrodes (Biosense Webster) with interelectrode distances of 2-5-2 mm were also used. When using the point-by-point method, at least 250 points taken at stable contact of the electrode with the endocardium were used to construct the LA map. The LA voltage charts were analyzed by an experienced electrophysiologist in the postoperative period. The LVZ area was estimated by the presence of 3 or more adjacent points with voltage<0.5 mV [12], and the total LVZ area was calculated as a percentage of the total LA area. It should be noted that the

area of the mitral valve and the mouths of the PV were excluded from the LA area. RFA was performed at a maximum power of 35–40 W, a temperature of 43 °C, and a flow rate of 7–30 mL/min. The end point of circumferential PV isolation was the entrance and exit block.

Statistical analyses

Statistical analysis of data was performed using IBM SPSS Statistics 21 and Statistica 12.0 programs. The distribution of quantitative variables was examined using the Shapiro-Wilk test. In case of normal distribution, data are presented as the mean (M) and standard deviation (M±SD), and in case of abnormal distribution, data are presented as median (Me) and interquartile range – Me (Q1; Q3). Comparisons of continuous variables between groups were made using the T-test in case of normal distribution and by the Mann-Whitney U test in case of other distribution. Qualitative data were compared by Pearson's $\chi 2$ test. Cut-off values of quantitative variables were determined by ROC analysis. Spearman correlation was used to analyze the relationship between serum markers and other characteristics.

The influence of variables on the onset of AF recurrence was assessed using freedom-from-arrhythmia curves according to Kaplan-Mayer analysis and single-factor Cox analysis. A predictive model of time to AF recurrence was constructed using multivariate Cox analysis. Results were considered statistically significant at $p<0.05$ and as having a tendency to significant differences at a level of $p<0.1$.

Results

Baseline characteristics in patients with and without AF recurrence

Of the 165 patients included in the prospective follow-up, 131 patients whose follow-up duration reached 18 months after the end of the blanking period (3 months after the date of RFA) were included in the final analysis. The median age of the patients was 59.0 (50.0; 64.0) years, and 80 patients (61%) were male. Paroxysmal AF was present in 103 (79%) and persistent AF in 28 (21%) patients.

During the 18 month follow-up, 47 patients (35.9%) had AF recurrences according to the primary endpoint criteria. Baseline characteristics and comorbidities according to presence of AF recurrence are presented in Table 1.

As shown in Table 1, among patients with late AF recurrence at baseline, there was a higher proportion of those with a history of AF lasting more than 1 year and those with early AF recurrences (during the blanking period). In the group with AF recurrences, there was a tendency to take propafenone more frequently and lappaconitine hydrobromide more rarely. It is important

Table 1 Patients' clinical and demographic characteristics according to presence of late AF recurrence

Characteristics	No AF recurrence (*n* = 84)	AF recurrence (*n* = 47)	*p*
Age, years	58.0 (49.5; 64.0)	60.0 (48.0; 64.0)	0.457
Male sex, n (%)	52 (61.9)	28 (59.6)	0.793
Current smoker, n (%)	10 (11.9)	4 (8.5)	0.276
BMI, kg/m^2	30.5 ± 4.4	30.0 ± 4.9	0.628
Obesity, n (%)	4 (53.6)	19 (40.4)	0.149
Hypertension, n (%)	67 (79.8)	43 (91.5)	0.079
Coronary artery disease, n (%)	30 (35.7)	23 (48.9)	0.139
Diabetes mellitus, n (%)	8 (9.5)	5 (10.6)	0.838
eGFR < 60 mL/min/1.73 m^2, n (%)	7 (8.4)	6 (13.0)	0.543
HF, n (%)	25 (29.8)	14 (29.8)	0.978
Type of AF, n (%):			
• Paroxysmal	64 (76.2)	39 (83.0)	0.363
• Persistent	20 (23.8)	8 (17.0)	
Duration of AF history ≥ 1 year	9 (10.6)	13 (27.7)	0.025
CHA$_2$DS$_2$VASc score, n (%):			
• 0	8 (9.5)	2 (4.3)	0.219
• 1	29 (34.5)	13 (27.7)	
• 2	28 (33.3)	16 (34.0)	
• > 2	19 (22.7)	16 (34.7)	
Early AF recurrence, n (%)	7 (8.3)	17 (36.2)	0.0005
Medications before RFA			
Amiodaron, n (%)	11 (13.1)	6 (12.8)	0.957
Propafenon, n (%)	11 (13.1)	12 (25.5)	0.073
B-Blocker, n (%)	25 (29.8)	11 (23.4)	0.434
Sotalol, n (%)	17 (20.2)	14 (29.8)	0.217
Lappaconitine hydrobromide, n (%)	14 (16.7)	3 (6.4)	0.093
Statin, n (%)	54 (64.3)	31 (67.4)	0.722
ARB and/or ACEi, n (%)	56 (66.6)	35 (76.1)	0.533
Diuretics, n (%)	23 (27.4)	13 (28.3)	0.915
Calcium antagonists, n (%)	10 (11.9)	10 (21.7)	0.137
Medications after RFA			
Amiodaron, n (%)	8 (9.5)	8 (17.0)	0.209
Propafenon, n (%)	9 (10.7)	9 (19.1)	0.179
B-Blocker, n (%)	23 (27.4)	8 (17.0)	0.181
Sotalol, n (%)	24 (28.6)	18 (38.3)	0.253
Lappaconitine hydrobromide, n (%)	10 (11.9)	5 (10.6)	0.827

Abbreviations: AF, atrial fibrillation; BMI, body mass index; MI, myocardial infarction; eGFR, estimated glomerular filtration rate; HF, heart failure; RFA, radiofrequency ablation; ARB, angiotensin receptor blocker; ACEi, angiotensin-converting enzyme inhibitor. *p* < 0.05 indicates significant difference

Table 2 Echocardiographic parameters, EAM data and biomarkers according to presence of AF recurrences

Characteristics	No AF recurrence (*n* = 84)	AF recurrence (*n* = 47)	*p*
LVEF, %	63.8 ± 6.2	63.6 ± 6.4	0.809
LVESD, mm/m^2	15.4 ± 2.7	15.4 ± 2.3	0.553
LVEDD, mm/m^2	23.9 ± 3.2	24.5 ± 2.6	0.099
LV mass, g/m^2	85.9 (75.6; 96.8)	92.1 (78.5; 112.0)	0.127
RAVi, mL/m^2	22.1 (16.8; 28.0)	23.5 (20.4; 31.0)	0.138
LAVi, mL/m^2	29.8 (23.5; 36.3)	31.6 (24.8; 37.3)	0.537
PASP, mm Hg	22.0 (19.0; 27.0)	23.0 (21.0; 27.0)	0.316
Septal e' velocity, cm/s	7.2 (6.0; 9.6)	7.2 (6.3; 9.0)	0.701
Later e' velocity, cm/s	10.0 (8.5; 12.0)	9.4 (7.9; 12.0)	0.365
E/e' ratio	7.1 (6.26; 9.17)	7.6 (5.85; 9.79)	0.455
LAAFV, cm/s	55.0 (45.0; 66.0)	52.0 (41.0; 56.0)	0.019
LAAFV > 54 cm/s, n (%)	43 (54.4)	15 (34.9)	0.039
LVZ area (%)	12.2 (4.8; 26.)	13.6 (4.8; 34.3)	0.537
LVZ area > 20%	27 (32.1)	20 (42.6)	0.233
NT-proBNP, pg/mL	87.9 (34.1; 194.0)	89.2 (35.1; 179.0)	0.998
NT-proBNP > 125 pg/mL	28 (37.8)	15 (35.7)	0.979
sST2, ng/mL	42.2 (29.8; 53.7)	44.1 (39.6; 50.9)	0.279
sST2:			
• < 36.0 ng/mL	29 (36.3)	8 (17.8)	0.030
• ≥ 36.0-48.4 ng/mL	25 (31.3)	22 (48.9)	0.051
• ≥ 48.5 ng/mL	26 (32.4)	15 (33.3)	0.924
sST2 ≥ 36 ng/mL	51 (63.7)	37 (82.2)	0.03
GDF-15, pg/mL	788.0 (630.0; 1030.5)	826.0 (634.0; 954.5)	0.939

Abbreviations: AF, atrial fibrillation; LVEF, left ventricular ejection fraction; LVESD, LV end-systolic dimension; LVEDD, LV end-diastolic dimension; RAVi, right atrial volume index; LAVi, left atrial volume index; PASP, pulmonary artery systolic pressure; LAAFV, left atrial appendage flow velocity; LVZ, low-voltage zones; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; sST2, soluble ST2; GDF-15, growth differentiation factor 15. *p* < 0.05 indicates significant difference

also lower. There were no significant differences in the LVZ area in patients with and without AF recurrences.

The medians of NT-proBNP, sST2, and GDF-15 in patients as a whole were as follows: 92.9 (40.9; 194.0) pg/mL, 43.3 (32.2; 51.2) ng/mL, and 813.0 (630.0; 988.0) pg/mL, respectively. The medians of all biomarkers did not differ significantly between the groups. At the same time, in patients with AF recurrences, the proportion of those with sST2 levels exceeding the threshold value of 36 ng/mL, which separated the lower and middle sST2 terciles, was significantly higher (Table 2).

Correlation between baseline biomarkers and other characteristics

The results of the correlation analysis are presented in Table 3. NT-proBNP and GDF-15 had a weak direct correlation with each other; however, neither showed any correlation with sST2. Both NT-proBNP and GDF-15 had a direct correlation with age, with a stronger correlation being observed for GDF-15. Only GDF-15 showed a

to note that the patients of both groups did not differ in antiarrhythmic therapy after RFA.

Among the TTE indicators in the group with AF recurrences, there was a tendency to a greater LV end-diastolic dimension (Table 2). The median LAAFV calculated in the group of patients as a whole was 54 (45.0; 59.0) cm/s. Moreover, the median LAAFV in the group with AF recurrences was significantly lower and the proportion of patients with a LAAFV value exceeding the median was

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Table 3 Correlation coefficients (r) in Spearman analysis between serum biomarkers and other characteristics

Parameters	NT-proBNP		GDF-15		sST2	
	r	p	r	p	r	p
Age	0.278	0.002	0.523	0.0001	0.023	NS
BMI	-0.103	NS	0.258	0.011	0.058	NS
Fasting blood glucose	0.021	NS	0.228	0.025	0.089	NS
LAVi	0.322	0.0001	0.121	NS	0.041	NS
RAVi	0.219	0.013	0.117	NS	0.190	0.021
LVEF	-0.218	0.012	-0.135	NS	-0.046	NS
LV mass index	0.193	0.027	0.213	0.036	0.053	NS
LAAFV	-0.140	NS	0.096	NS	0.013	NS
LVZ area	0.314	0.0001	0.247	0.016	0.091	NS
NT-proBNP	–	–	0.224	0.028	-0.008	NS
GDF-15	0.224	0.028	–	–	0.033	NS
sST2	-0.008	NS	0.033	NS	–	–

Abbreviations: BMI, body mass index; LAVi, left atrial volume index; RAVi, right atrial volume index; LVEF, left ventricular ejection fraction; LAAFV, left atrial appendage flow velocity; LVZ, low-voltage zones; NT-proBNP, N-terminal pro-brain natriuretic peptide; sST2, soluble ST2; GDF-15, growth differentiation factor 15; r, correlation coefficient; NS, non-significant. $p < 0.05$ indicates significant difference;

weak direct correlations with body mass index and fasting blood glucose levels.

NT-proBNP had weak positive correlations with indexed volumes of both the right (RAVi) and left atria (LAVi) as well as left ventricular mass index (LVMMi), but weak negative correlation with LVEF. GDF-15 was weakly positively correlated with LVMMi; however, there was no significant correlation with the indexed volumes of both atria. sST2 had a weak significant positive correlation only with RAVi. There was no correlation of the sST2 level with LAVi, despite the presence of a moderate direct correlation between RAVi and LAVi ($r = 0.663$, $p = 0.0001$). None of the biomarkers had a significant correlation with LAAFV. Both NT-proBNP and GDF-15 had a weak positive correlation with LVZ, unlike sST2.

The medians of all three biomarkers were significantly lower in paroxysmal AF compared to persistent AF, which were 84.9 (41.8; 184.0) and 418.5 (144.5; 1078.0) pg/mL ($p < 0.0001$), respectively, for NT-proBNP; 828 (671.5; 1034.5) and 984,9 (739.3; 1440.8) pg/mL ($p < 0.01$), respectively, for GDF-15; and 42.4 (30.7; 50.6) and 46.8 (33.4; 61.9) ng/mL ($p < 0.01$), respectively, for sST2. Only for sST2 was there a tendency towards higher median levels when AF history was more than 1 year: 51.1 (33.0; 56.7) ng/mL vs 43.1 (31.6; 50.9) ng/mL ($p = 0.0969$).

Results of prospective follow-up and search for AF recurrence predictors

The independent variables affecting the primary endpoint, considering the time of AF recurrence, were analyzed using the Kaplan-Mayer method and Cox proportional hazard regression. Time to AF recurrence was taken as the dependent variable while clinical and echocardiographic parameters, EAM data, and biomarkers were used as independent variables. When the Kaplan-Mayer survival curves were compared, the incidence of late AF recurrences was found to be significantly higher in groups with AF history ≥ 1 year, early AF recurrences, LAAFV < 54 cm/s, and sST2 level ≥ 36 ng/mL (Fig. 1.).

Using single-factor Cox regression analysis, the extent to which these variables and other potential risk factors identified in previous studies influenced the likelihood of late AF recurrence was examined (Table 4). In the next step, these potential predictors of long-term AF recurrence were included in the multivariate Cox analysis. Using the Backward-method, two independent predictors of late AF recurrences after primary RFA were obtained: sST2 level ≥ 36 ng/mL and LAAFV < 54 cm/s (Table 4).

It should be emphasized that sST2 level was better at predicting long-term AF recurrences than the persistent form of AF; however, the form should be considered since the median sST2 in persistent AF was higher ($p < 0.05$) than in paroxysmal AF. This proves that increased levels of the proinflammatory marker sST2 have a significant relationship with the pathophysiological mechanisms underlying AF recurrence in long-term period after RFA.

Discussion

Predicting long-term AF recurrence after primary RFA remains an important task not only to identify optimal patients for this treatment procedure, but also to find new additional targets for exposure to the AF substrate, as the efficacy of catheter ablation has been shown to be substrate-dependent, with inflammation and fibrosis being the primary underlying factors [13].

The existing prognostic scales or separate clinical, laboratory, and echocardiographic indicators are not sufficiently specific for this category of patients, do not have high prognostic significance [14], and do not consider the further progression of the AF substrate within a single AF continuum. From this point of view, the inclusion of

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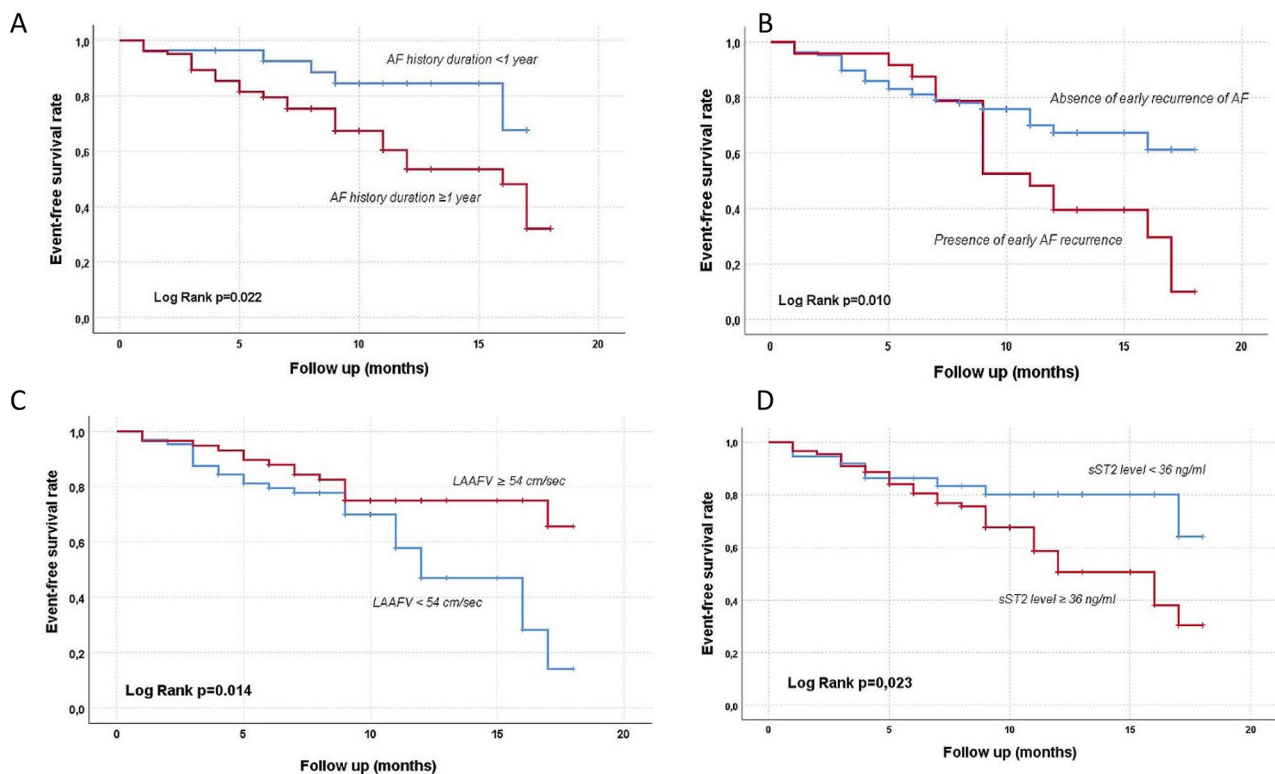
Fig. 1 Kaplan-Meier curves of event-free survival rate as a function of four variables: **A** – duration of AF history; **B** – presence of early AF recurrence; **C** – LAAFV; and **D** – sST2 level. Legends: **A** – the AF history duration (blue – <1 year, red – ≥1 year); **B** – presence of early AF recurrence (blue – no, red – yes); **C** – LAAFV (blue – <54 cm/s, red – ≥54 cm/s); **D** – sST2 level (blue – <36 ng/mL, ≥ 36 ng/mL)

Table 4 Results of univariate and multivariate Cox regression analysis on characteristics related to AF recurrence after RFA

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age (years)	1.015	0.985–1.045	0.337	–	–	–
Sex (male)	0.854	0.426–1.714	0.658	–	–	–
AF history ≥ 1 year	2.761	1.089–6.998	0.032	–	–	–
Early AF recurrence	2.105	1.158–3.828	0.015	–	–	–
Paroxysmal AF	0.471	0.180-1,232	0.125	–	–	–
LAVi (mL/m^2)	1.004	0.975–1.033	0.804	–	–	–
RAVi (mL/m^2)	1.019	0.963–1.078	0.516	–	–	–
LVZ area > 20%	1.554	0.866–2.789	0.139	–	–	–
sST2 ≥ 36 ng/mL	2.327	1.078–5.023	0.031	3.810	1.477–9.830	0.006
LAAFV < 54 cm/s	2.141	1.134–4.043	0.019	1.961	1.006–3.822	0.048

Abbreviations: CI, confidence interval; AF, atrial fibrillation; LAVi, left atrial volume index, RAVi, right atrial volume index; LVZ, low-voltage zones; sST2, soluble ST2; LAAFV, left atrial appendage flow velocity; HR, hazard ratio; CI, confidence interval

inflammation and fibrosis biomarkers in the list of potential predictors of AF recurrences seems appropriate.

In our study, four variables that affect the time of the first AF recurrence in the long-term period after primary RFA were identified. Our study confirmed the well-known fact that early recurrences are themselves predictors of late AF recurrences [1, 2]. It is believed that the cause of early recurrences is incomplete isolation or inflammation as a result of exposure to ablative energy.

Isolation of PV mouths, separating AF triggers from the surrounding atrial tissue, has maximum efficacy in the early stages of the AF development, which was confirmed in our study by the fact that the second factor associated with AF recurrence was the AF history duration of 1 year or more. This variable was not saved in the list of independent predictors; however, it should be noted that, of the studied biomarkers, only sST2 level was associated with the AF history duration: there was a tendency to a higher level in patients with an AF history

duration of more than 1 year. On the other hand, it can be assumed that the AF substrate progression substrate depends more on the intensity of inflammation than on its duration.

As for the association of a lower LAAFV with AF recurrences, this is consistent with the results of the study of Suksaranjit P. et al. [15], which found that the extent of LAA structural remodeling identified by LGE-MRI is associated with arrhythmia recurrence after AF ablation. There was an approximately four-fold increase in the risk of arrhythmia recurrence (adjusted HR 4.12, 95% CI 1.26–13.46) in patients with advanced LAA structural remodeling (T4 vs T1) [15]. According to our results, LAAFV was weakly negatively correlated with LVMMi (r = -0.196, p=0.015), LAVi (r = -0.211, p=0.009), and LVZ area (r = -0.181, p=0.025). Thus, LAAFV manifests itself as an indicator, which is influenced not only by the structural and functional remodeling of the left atrium, but also of the left ventricle.

Our choice of biomarkers for the study was dictated by the available evidences for their prognostic significance in relation to the development, progression, and outcomes of AF [4–6, 16, 17], as well as our previous pilot studies showing the association of NT-proBNP and GDF-15 with LVZ area [7, 8].

Correlation analysis showed a direct relationship of NT-proBNP and GDF-15 with each other, with age, as well as with the parameters of structural and functional remodeling of both atria and left ventricle, and LVZ area, whereas sST2 was associated only with RAVi and was independent of age. We cannot explain the relationship of sST2 with the indexed right atrium volume but not the left atrium volume, although the indexed volumes of both atria significantly correlated with each other. The most likely explanation for this is the limited sample size and the abnormal distribution of data. Nevertheless, it is believed that RA may be involved in the pathogenesis of AF at earlier stages than LA [18].

According to our results, NT-proBNP and GDF-15 were not among the independent predictors of AF recurrence, unlike sST2. In contrast to our results, Ying Wei et al. in a prospective study of 150 patients with AF (mean age 64±11 years, median follow-up of 14.0 months) determined that increased preprocedural GDF-15 is associated with left atrial remodeling and acts as a predictor of AF recurrence after RFA [19]. The differences between the above results and ours may be due to the fact that the patients in Wei et al.'s study were older, 41% of them had persistent AF (41%), and 35% had diabetes mellitus [20–22]. This is confirmed by the correlation we obtained between GDF-15 and fasting blood glucose levels.

We can assume that NT-proBNP and GDF-15 are closer related to the size of the AF substrate at the time of the prognostic assessment, while the prognostic capabilities of sST2 in the long-term prediction of further substrate progression are higher.

The Okar S. et al. [23] was the first to show an association between sST2 and AF recurrence after cryoballon ablation. In this study, sST2 was the only independent parameter for predicting AF recurrence: every 10-unit increase in sST2 was found to be associated with a 2.10-fold increase in the risk of AF recurrence. The authors concluded that the sST2 level, which is associated with atrial fibrosis, can be considered a useful marker for identifying patients with high-grade fibrosis who will benefit less from cryoablation [23].

A biomarker closely related to fibrosis and inflammation, sST2 plays an important role in the pathogenesis and progression of AF, and also has certain value in predicting the occurrence, progression, recurrence and prognosis of AF [24]. It was established that the effect of sST2 may be associated with the inhibition of the IL-33/ST2l signaling pathway: sST2 is a receptor for IL-1 and competitively binds to IL-33, suppressing the protective effect of IL-33/ST2l on the myocardium [24, 25]. When myocardium induced by pressure or volume overload produces large amounts of sST2, high concentrations of sST2 interfere with the effects of IL-33/ST2l and may therefore lead to atrial fibrosis [26]. This assumption is completely consistent with the results of the prospective study by Liu H. et al. [3] evaluating the role of sST2 in predicting AF recurrence in 258 patients (including 37.5% with persistent AF) after RFA (median follow-up 13.5 months). The study is notable for the fact that EAM was repeated in the long-term period due to recurrent AF. Despite the differences in the baseline sST2 level in patients with and without AF recurrence, the initial sST2 level did not depend on the LVZ area determined during primary EAM. At the same time, in patients who had an increase of the LVZ area during repeated RFA, which confirmed the progression of the substrate, the level of sST2 was significantly higher than in patients whose substrate sizes remained the same. The authors concluded that elevated plasma sST2 levels indicate progressive atrial fibrosis and is an important prognostic factor for AF recurrence after RFA.

Similar to the results of the study by Liu H. et al., we did not obtain a link between the baseline sST2 level and the LVZ area, but during follow-up for 18 months in patients with sST2 levels≥36 ng/mL late AF recurrences occurred earlier. This finding supports the view that sST2 has notable prognostic performance, but low diagnostic performance [27]. It is likely that the sST2 level above the cut-off value largely reflects the pro-inflammatory and profibrotic potential for further substrate progression, which will be realized in the future. Thus, it can be assumed that after RFA, inflammation continues with

the progression of the AF substrate, which is the cause of long-term recurrence, despite the high-quality PV isolation. This explains the fact that the sST2 level exceeded the LVZ area as a predictor, since the initial LVZ area has less effect on the outcomes of RFA than ongoing inflammation.

We believe that the results obtained provide a basis for continuing research to develop a personalized approach to choosing an AF treatment strategy based on determining the baseline level of sST2.

We assume that in patients with initially elevated sST2 levels, it is necessary to evaluate the possibilities of a combined treatment approach using catheter ablation and drugs with anti-inflammatory and antifibrotic effects.

Limitations

The study was single-center. The overall sample size of this study is small, so the power is not strong enough and the capabilities of sST2 in predicting long-term AF recurrence need further study.

When determining the level of GDF-15, an analytical research kit was used, which dictates the need to expand the scope of the study, as well as the introduction of control groups of pts to determine reference values, including for certain age categories.

Although we applied the ECG recording system in this study, subclinical AF could also occur, and thus its impact on the outcome is unavoidable.

The lack of confirmation of the size of the LVZ area as predictors of late AF recurrence may probably be due to the fact that EAM in some pts was carried out using the «point-by-point» method.

Conclusion

Serum sST2 level with a cut-off value of 36 ng/ml or more can be used as a predictor of AF recurrence in the long-term period after primary RFA. This can play an important role in a personalized approach to choosing treatment strategies in patients with AF.

Abbreviations

AF	Atrial fibrillation
RFA	Radiofrequency ablation
PV	Pulmonary veins
IL-1	Interleukin-1
sST2	Soluble suppression of tumorigenicity 2
LVZ	Low-voltage zones
LA	Left atrium
EAM	Electroanatomic mapping
GDF-15	Growth differentiation factor 15
LAA	Left atrial appendage
TEE	Transesophageal echocardiography
LVEF	Left ventricular ejection fraction
ECG	Electrocardiogram
TTE	Transthoracic echocardiography
NT-proBNP	N-terminal pro-brain natriuretic peptide
LV	Left ventricular
LAAFV	Left atrial appendage flow velocity
LVMMi	Left ventricular myocardial mass index
LAVi	Left atrial volume index
RAVi	Right atrial volume index
LGE-MRI	Magnetic resonance imaging with delayed gadolinium

Declarations

Ethics approval and consent to participate
This study adhered to the guidelines outlined in the revised 2013 Helsinki Declaration, and informed consent was obtained from all patients individually. The study protocol received approval from the Scientific Ethics Committee of the Tyumen Cardiology Research Center (protocol code 153 and 02/10/2019). We followed all relevant guidelines and regulations during the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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