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ATRIAL FIBRILLATION AND CHANGE OF EXTRACELLULAR MATRIX

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Summary

The objective: to investigate the changes of serum markers of collagen in patients with various forms of atrial fibrillation (AF). **Methods:** C-terminal propeptide of type I collagen (CTTP-I), C-terminal telopeptide of type I collagen (CTTC-I), matrix metalloproteinase-I, and tissue inhibitors of matrix metalloproteinase I were used as markers of collagen synthesis. The study group included 70 persons, the control one — 20. **Results:** The levels of CTPC-I and CTTK-I in patients with AF were significantly higher compared with the control group (91 ± 27 ng / ml., 67 ± 11 ng / ml, $p < 0.001$ and 0.38 ± 0.20 ng / ml, 0.25 ± 0.08 ng / ml, $p < 0.001$, respectively). The level of CTPA-I in patients with persistent and chronic forms of AF was significantly higher (105 ± 28 ng / ml and 126 ± 26 ng / ml versus 80 ± 21 ng / ml, $p < 0.001$) compared with paroxysmal AF patients. Patients with persistent and chronic forms of AF showed significantly lower level of matrix metalloproteinase-1 (MMP-1), however, the level of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) was increased in comparison with patients with paroxysmal AF (09.67 ± 4.41 ng / ml, 11.90 ± 4.79 ng / ml against 14.98 ± 6.28 ng / ml, p = 0.03 and 187 ± 49 ng / ml, 155 ± 45 ng / ml against 130 ± 38 ng / ml, $p < 0.001$, respectively). The level of TIMMP-1 was significantly lower in the control group compared with patients with paroxysmal, persistent and chronic forms of AF (102 ± 15 ng / ml, 130 ± 38 ng / ml, 155 ± 45 ng / ml and 194 ± 49 ng / ml, respectively, $p < 0.001$). **Conclusions:** Serum levels of type I collagen markers are significantly different between healthy people and patients with AF. Moreover, these markers also differ depending on the form of AF. It can be assumed that the intensity of extracellular synthesis and degradation of type I collagen may be related to the severity and type of AF.

Key words: **serum markers of collagen, atrial fiblillation, extracellural syntheis.**

ЗМІНА ПОЗАКЛІТИННОГО МАТРИКСУ У ПАЦІЄНТІВ З РІЗНИМИ ФОРМАМИ ФІБРИЛЯЦІЇ ПЕРЕДСЕРДЬ

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Резюме

Мета: нами було досліджено зміну сироваткових маркерів колагену у пацієнтів з різними формами фібриляції передсердь (ФП). **Методи:** Як маркери синтезу колагену використовувалися С-термінальний пропептид колагену I типу (ЦТПК-I), С-термінальний телопептид колагену I типу (ЦТТК-I), матриксна металлопротеіназа-I, і тканинні інгібітори матриксної металлопротеінази I. Досліджувану групу склали — 70 осіб, контрольну — 20.

Результати: Рівень ЦТПК-I і ЦТТК-I у пацієнтів з ФП був значно вище в порівнянні з контрольною групою (91 ± 27 нг/мл., 67 ± 11 нг/мл, $p < 0,001$ і 0,38 ± 0,20 нг/мл, 0,25 ± 0,08 нг/мл, $p < 0,001$, відповідно). Рівень ЦТПК-I у пацієнтів з персистуючою та хронічною формами ФП був значно вище (105 ± 28 нг/мл і 126 ± 26 нг/мл проти. 80 ± 21 нг/мл, $p < 0,001$) в порівнянні з пацієнтами з пароксизмальною формою ФП. У пацієнтів з персистуючою та хронічною формами ФП відзначався значно нижчий рівень матриксної металлопротеінази-1 (ММП-1) проте рівень тканинного інгібітора матриксної металлопротеінази-1 (ТІММП-1) був підвищений в порівнянні з пацієнтами з пароксизмальною ФП (09,67 ± 4,41 нг/мл, 11.90 ± 4,79 нг/мл проти 14,98 ± 6,28 нг/мл, p = 0.03 і 187 ± 49 нг/мл, 155 ± 45 нг/мл проти 130 ± 38 нг/мл, $p < 0.001$, відповідно). Рівень ТІММП-1 був значно нижче в контрольній групі в порівнянні з пацієнтами з пароксизмальною, персистуючою і хронічною формами ФП (102 ± 15 нг/мл, 130 ± 38 нг/мл, 155 ± 45 нг/мл і 194 ± 49 нг/мл, відповідно, $p < 0,001$). **Висновки:** Сироватковий рівень маркерів колагену I типу значно відрізняється між здоровими людьми та пацієнтами з ФП. Більш того ці маркери також відрізняються в залежності від форми ФП. Можна припустити, що інтенсивність позаклітинного синтезу і деградації колагену I типу може бути пов'язана з тяжкістю і типом ФП.

Ключові слова: сывороточный маркер коллагена, фибрилляция предсердий, клеточный синтез.

ИЗМЕНЕНИЕ ВНЕКЛЕТОЧНОГО МАТРИКСА У ПАЦИЕНТОВ С РАЗЛИЧНЫМИ ФОРМАМИ ФИБРИЛЛЯЦИИ ПРЕДСЕРДИЙ

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Резюме

Цель: нами было исследовано изменение сывороточных маркеров коллагена у пациентов с различными формами фибрилляции предсердий (ФП). *Методы:* В качестве маркеров синтеза коллагена использовались С-терминальный пропептид коллагена I типа (ЦТПК-I), С-терминальный телопептид коллагена I типа (ЦТТК-I), матриксная металлопротеиназа- I, и тканевые ингибиторы матриксной металлопротеиназы I. Исследуемую группу составили — 70 человек, контрольную — 20. **Результаты:** Уровень ЦТПК-I и ЦТТК-I у пациентов с ФП был значительно выше по сравнению с контрольной группой (91 ± 27 нг/мл., 67 ± 11 нг/мл, $p < 0{,}001$ и 0.38 ± 0,20 нг/мл, 0,25 ± 0,08 нг/мл, $p < 0{,}001$, соответственно). Уровень ЦТПК-I у пациентов с персистирующей и хронической формами ФП был значительно выше (105 ± 28 нг/мл и 126 ± 26 нг/мл против. 80 ± 21 нг/мл, $p < 0{,}001$) по сравнению с пациентами с пароксизмальной формой ФП. У пациентов с персистирующей и хронической формами ФП отмечался значительно более низкий уровень матриксной металлопротеиназы-1 (ММП-1) однако уровень тканевого ингибитора матриксной металлопротеиназы-1 (ТИММП-1) был повышен в сравнении с пациентами с пароксизмальной ФП (09.67 ± 4.41 нг/мл, 11.90 ± 4.79 нг/мл против 14.98 ± 6.28 нг/мл, p = 0.03 и 187 ± 49 нг/мл, 155 ± 45 нг/мл против 130 ± 38 нг/мл, $p < 0{,}001$, соответственно). Уровень ТИММП-1 был значительно ниже в контрольной группе в сравнении с пациентами с пароксизмальной и персистирующей и хронической формами ФП (102 ± 15 нг/мл, 130 ± 38 нг/мл, 155 ± 45 нг/мл и 194 ± 49 нг/мл, соответственно, $p < 0{,}001$).

Выводы: Сывороточный уровень маркеров коллагена I типа значительно отличается между здоровыми людьми и пациентами с ФП. Более того эти маркеры также отличаются в зависимости от формы ФП. Можно предположить, что интенсивность внеклеточного синтеза и деградации коллагена I типа может быть связанна с тяжестью и типом ФП.

Ключевые слова: сывороточный маркер коллагена, фибрилляция предсердий,

клеточный синтез.

Background. Recent studies have shown a significant increase in the level of collagen deposition in the atria in patients with AF, unlike patients who are on sinus rhythm (SR) [1-3]. Preliminary experimental and clinical data suggested a feedback between the presence of fibrosis in biopsy samples and the presence of arterial hypertension (AH). It was further shown that such markers differ in patients with hypertension and without it, studies of their connection with hypertrophy or other echocardiographic parameters have also led to contradictory [5,6].

Collagen type I is the most common collagen product of cardiac fibroblasts [7]. We have estimated the amount of fibrosis in patients with paroxysmal, persistent and chronic isolated forms of AF using enzyme immunoassay [4]. The level of MMP-I and TIMP-I was also evaluated.

Materials and methods. The study was approved by the ethical committee of the Odessa Regional Hospital. It was consistent with the principles set forth in the Helsinki Declaration. All patients signed an informed consent to participate in it.

96 outpatient patients aged 24 — 78 y. o. with isolated AF were included into it with AF without clinical or echocardiographic signs of cardiopulmonary disease, including AH. Arrhythmia was considered to be paroxysmal with a duration of less than 24 hours and a persistent duration of at least 3 months until the moment of inclusion. Chronic form of AF was called a rhythm disorder for more than one year, resistant to drug therapy. Forms of AF were determined according to the leadership standard of the European Society of Cardiology, 2016 [8]. The control group consisted of 24 patients with no history of AF.

Exclusion criteria: conditions associated with elevated serum marker of myocardial or tissue fibrosis, such as liver disease, renal dysfunction, pulmonary fibrosis, extensive wound surfaces, metabolic bone diseases, malignant neoplasms, connective tissue diseases, chronic inflammatory diseases, recently suffered infectious diseases and surgical interventions, age over 80 y.o. or presence of an implanted pacemaker / implantable cardioverter defibrillator (ICD).

During the study, in the patients of the study and control groups serological markers of type I collagen, the echocardiographic size of the LA and the LA ejection fraction (LAEF) were compared. To control the frequency of ventricular contractions, diltiazem and beta-blockers were used. All AF patients received antithrombotic treatment.

At the time of blood sampling, all patients had AF. Blood samples were collected during a clinical trial and immediately placed on ice and centrifuged for 1 hour. Samples were stored at -80 ° C until analysis.

The serum levels of TIMP-1 and MMP-1 were evaluated using enzyme immunoassay

with laboratory kits (Human Biotrack, Amersham Biosciences, USA). The level of CTPK-I type was determined using enzyme immunosorbent assay with kit (Metra CICP, Quidel, USA), while CTTC-I was measured using an Elecsys B-CrossLaps / serum assay (Roche Diagnostics, Mannheim, Germany). The measurement was performed by staff blinded to the clinical information about the patients' condition. Inside and inter-test coefficients of variation were < 8 % and < 10 %, respectively.

Statistical analysis. The data obtained were processed statistically using the Statistica 6.1 computer program. Quantitative signs with a normal distribution are presented as M ± σ (mean ± standard deviation), with an abnormal distribution — in the form of a median and interquartile range (Me). To identify the existing differences in ordinal characteristics, the non-parametric Mann-Whitney test was used. Correlation analysis was performed using the Spearman *R* test for quantitative values. At $p < 0.05$, differences were considered statistically significant. The study design is represented by an open controlled one.

The results.

Patients. The initial clinical and demographic characteristics of the study population are presented in Table 1. Group I comprised 24 patients with paroxysmal AF, Group II — 26 patients with persistent AF, Group III — 22 patients with chronic AF and Group IV (control) — 24 AF-free patients. There were no significant differences in gender (p = 0.40) or age (p = 0.058) between the AF groups and the control group. Patients with persistent and chronic form of AF had lower levels of left ventricular ejection fraction (LVEF) (p = 0.038) and larger sizes of LA ($p < 0.001$) compared with patients with paroxysmal AF and the control group.

Serum collagen markers. The results obtained are shown in Table 1.

Table 1

GENERAL CHARACTERISTICS OF PATIENTS

	I gr., *n* = 24	II gr., *n* = 26	III gr., *n* = 22	IV gr., *n* = 24	p
Age, y.o.	62.45 ± 13.17	64.44 ± 13.81	67.88 ± 13.31	63.65 ± 13.34	0.025
Sex, m/f	16/8	19/7	15/7	17/7	0.621
LVEF, %	55.39 ± 3.32	53.65 ± 3.27	51.65 ± 3.27	60.09 ± 3.2	0.227
LA, mm	36.23 ± 3.87	43.47 ± 4.52	45.57 ± 4.75	37.45 ± 3.68	< 0.001
BMI, kg/m2	27.32 ± 1.64	27.65 ± 1.73	27.91 ± 1.71	26.85 ± 1.42	0.919
SBP, mm Hg	137.35 ± 11.90	138.59 ± 12.52	138.59 ± 12.52	131.75 ± 10.03	0.079
DBP, mm Hg	84.74 ± 4.34	83.91 ± 4.35	83.91 ± 4.35	83.75 ± 4.55	0.633
CTPC-I, ng/ml	78.74 ± 20.13	104.05 ± 26.47	109.76 ± 31.37	68.31 ± 12.16	< 0.001
CTTC-I, ng/ml	0.36 ± 0.17	0.40 ± 0.19	0.44 ± 0.21	0.27 ± 0.11	0.016
MMP-I, ng/ml	13.89 ± 5.34	11.87 ± 4.79	10.90 ± 4.91	12.53 ± 6.16	0.033
TIMP-I, ng/ml	131.51 ± 36.92	149.63 ± 43.71	155.90 ± 45.86	103.61 ± 14.96	< 0.001

Values are expressed as mean ± SD. Subsequent analysis showed that, 1) the age and diameter of LA differed significantly between control patients and patients with persistent AF; 2)

CTPC-I in persistent AF patients differed significantly compared with patients with AF paroxysmal form and control group patients (Fig. 1).

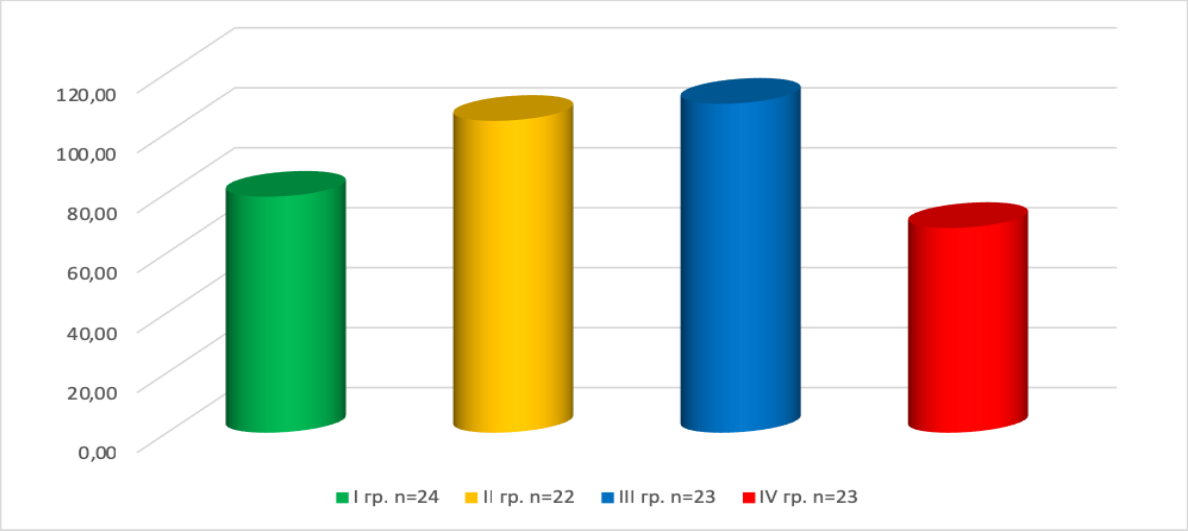

Fig. 1. The level of CTPC-I in patients with AF.

3) the level of CTTC-I was significantly different in patients of the control group and patients with various forms of AF (Figure 2);

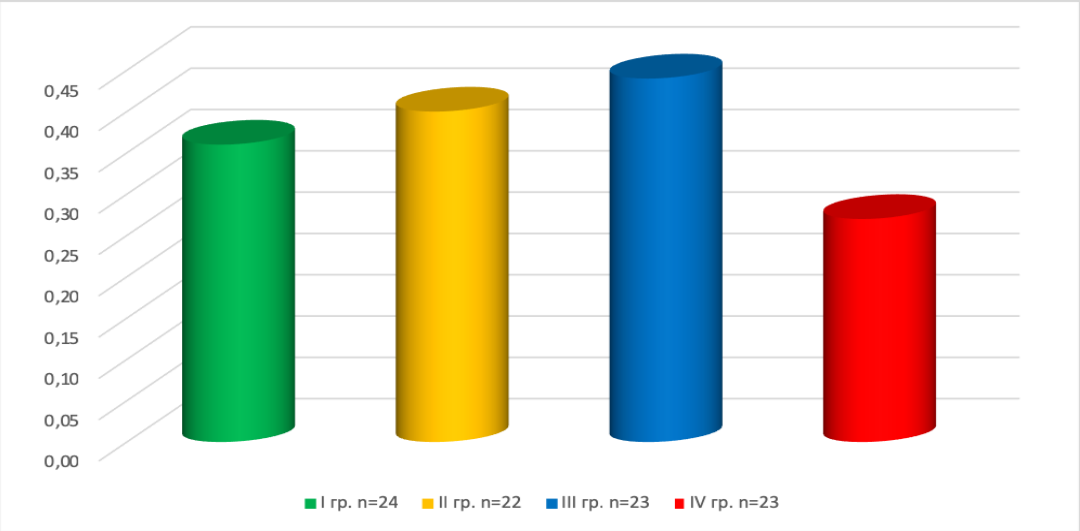

Fig. 2. The level of CTTK-I in patients with AF

4) the level of MMP-1 was significantly different in patients with persistent, chronic and paroxysmal forms of AF (Figure 3);

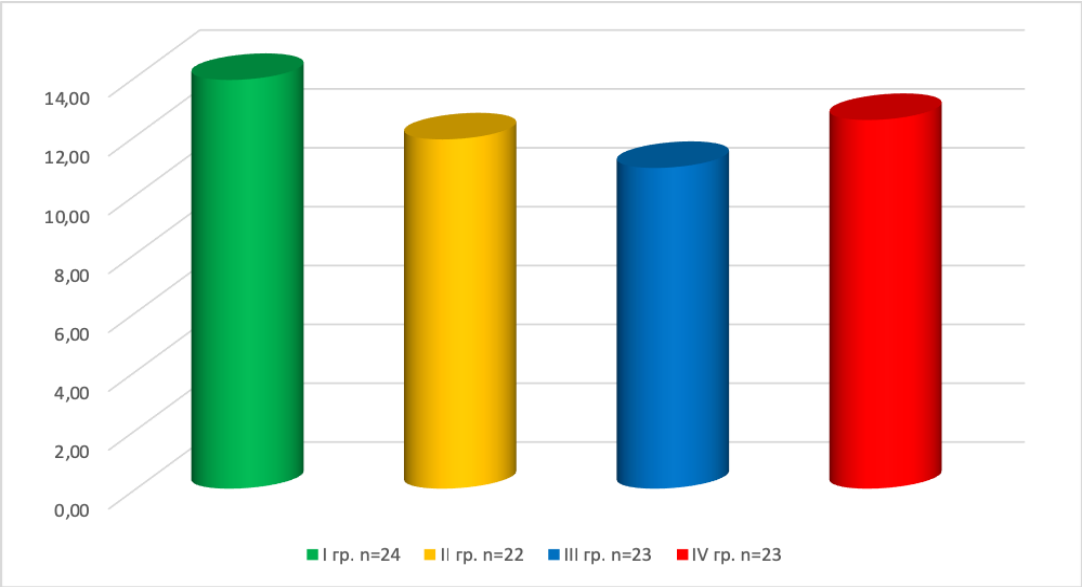

Fig. 3. The level of MMP-1 in patients with AF.

And 5) the level of TIMP-1 was significantly different in all intergroup comparisons (Figure 4).

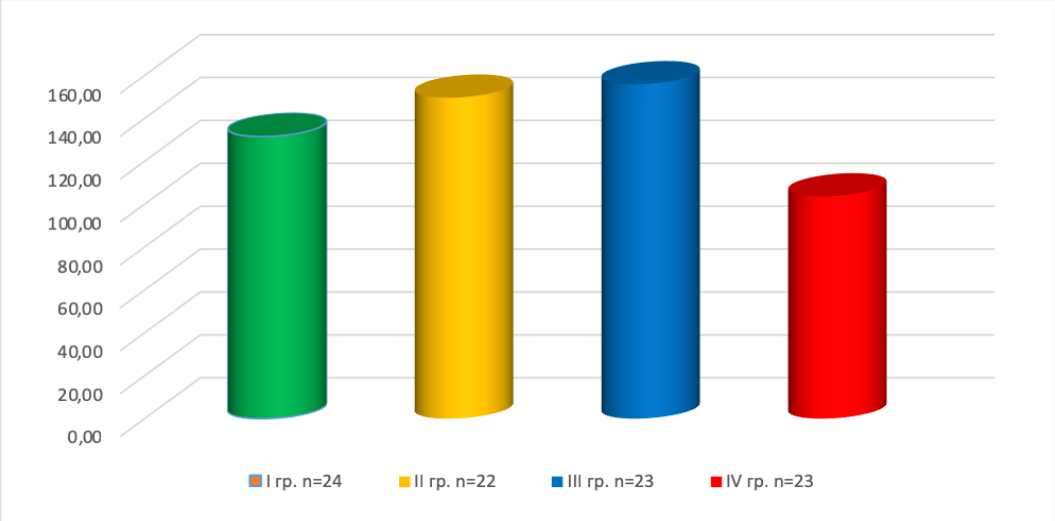

Fig. 4. TIMP-1 level in patients with AF.

Patients with persistent and chronic forms of AF had lower levels of MMP-1 ($p = 0.026$), but higher levels of TIMP-1 ($p = 0.013$) compared with patients with paroxysmal AF (Table 1, Fig. 3, 4). Tissue inhibitor of matrix metalloproteinases-1 was significantly lower ($p < 0.001$) in control subjects as compared with the studied patients (Fig. 4). The plasma MMP-1 level did not differ significantly between the studied patients of different groups and the control group (Fig. 3).

Finally, in all AF patients, taken together, a positive correlation between the levels of CTPC-I, TIMP-1 and the size of the LA ($r = 0.635$, $p < 0.001$ and $r = 0.563$, $p < 0.001$,

respectively) (Fig. 5), was observed, whereas the relationship between the levels of CTPC-I, TIMP-1 and LVEF is weakly expressed (r = -0.234, p = 0.05, br = -0.278, p = 0.020, respectively). A positive correlation was observed between the levels of MMP-1 and LVEF (r = 0.30, p = 0.012), whereas there was an inverse relationship between the levels of MMP-1 and the size of the LA (r = -0.615, $p < 0.001$). Logistic regression analysis showed that age, sex, size of the LA, the level of collagen markers TIMP-1, MMP-1, age and CTPC-I are associated with the presence of AF (Table 2).

Table 2

STEP-BY-STEP LOGISTIC REGRESSION ANALYSIS, PREDICTORS OF AF

Variable	Ratio	95 % confidence interval (CI)	
TIMP-I	1.18	1.07-1.29	0.002
MMP-I	1.76	1.27-2.27	0.001
Age	1.16	1.06-1.34	0.005
CTPC-I	1.07	1.003-1.14	0.037

Discussion

Patients who initially have paroxysmal AF often progress to persistent AF and ultimately the process becomes chronic. Although the exact pathophysiological mechanisms are poorly understood, it is believed that persistent AF arises from atrial remodeling [9,10]. However, pure electrical remodeling cannot explain the development of sustainable AF [10,11]. Atrial fibrosis may be involved as a factor with a slower course involved in this process [3, 12, 13].

In this study, we demonstrated an increase in CTPC-I and CTTC-I in the group of patients with AF, taken as a whole and compared with patients on SR. Interestingly, that patients with persistent and chronic forms of AF had the highest serum CTPC-I concentrations, whereas patients with persistent, chronic, and paroxysmal AF had no differences in CTTC-I levels. Thus, CTPC had a gradual growth from the control group to the group of patients with paroxysmal, persistent and then to the permanent form of AF was demonstrated; however, this connection was not observed in CTTC-I, presumably, the intensity of extracellular degradation of collagen type I was insufficient to compensate for it increased synthesis, which led to an increase in fibrosis in patients with persistent and chronic forms of AF.

In addition, in patients with chronic and persistent forms of AF, the levels of MMP-1 decreased, while levels of TIMP-1 increased compared with paroxysmal AF patients. The level of TIMP-1 was also higher in patients with paroxysmal AF than in the control group. In addition, a lower level of MMP-1 was observed in the control group than in paroxysmal AF, but higher than in patients with persistent and persistent forms of AF (although the differences did not reach

statistical significance). This seems paradoxical, but it can be the result of the activation of MMP-1, which depends on the nature of the stimulus and differs in acute and chronic stimulation [14]. Thus, paroxysmal AF can lead to a sharp overload of pressure or volume, activating the MMP-1 system, which is then compromised by prolongation and stabilization of the stimulus.

Another interesting finding was that the levels of CTPC-I and TIMP-1 correlated positively with the diameter of the left ventricle and are inversely related to LVEF, whereas in patients with AF with larger sizes of LV and lesser LVEF, it is likely that the arrhythmia is longer there were lower levels of MMP-1.

Since only patients with isolated AF were involved in our study, we can assume that the above mentioned changes were associated with the arrhythmia itself, and not with the presence or absence of any concomitant factor with a progressive increase in fibrosis from paroxysmal to chronic AF. In addition, enhanced fibrosis, especially in patients with the chronic form of AF, may also be causative in both initiating and maintaining AF.

Study limitations and clinical implications. Collagen serum markers are not specific for the heart. In addition, we did not confirm our data using atrial tissue biopsy data or coronary sinus sampling. However, we have made every effort to exclude subjects with conditions associated with the formation of fibrosis from the study.

Serial measurements of collagen indices after SR recovery for assessing potential temporal changes in collagen levels are not available, although these data would certainly be a valuable addition to our study and could confirm our results.

And finally, a small sample of patients does not allow to draw serious conclusions regarding the relationship between systemic fibrosis and AF. So, further research is needed.

Conclusions.

Serum markers of type I collagen can provide a non-invasive method of documenting and monitoring the extent and mechanisms of myocardial fibrosis in patients with AF, as well as assessing pharmacological measures for treating this arrhythmia. However, it is necessary to further study and conduct randomized studies to determine the exact role of fibrosis in the formation of AF and assess the clinical importance and value of biochemical monitoring of the level of collagen in this clinical situation. Although cardiac biopsy is the gold standard for documenting and monitoring myocardial fibrosis, non-invasive methods offer an alternative view of solving this problem, which may be more widely used.

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