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Early diagnosis of myocardial fibrosis in patients with epicardial obesity

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It has been proven that about half of patients with heart failure (HF) have a preserved the left ventricle (LV) ejection fraction (EF), which complicates early detection of HF. Currently, there is a search for non-invasive methods for diagnosing myocardial fibrosis at the preclinical heart failure to prevent its progression and the appearance of clinical symptoms.

Aim. To study the relationship of LV mechanics with the level of serum myocardial fibrosis markers in patients with epicardial obesity (EO).

Material and methods. The study included 110 men with general obesity. Depending on echocardiographic data, the patients were divided into 2 groups: EO (+) with epicardial fat thickness (EFT) ⩾7 mm (n=70); EO (-) with EFT <7 mm (n=40). All patients were studied for serum profibrotic markers (MMP-3, collagen I, collagen III, TGF-β, VEGFA, PICP) using enzyme-linked immunosorbent assay. Speckle-tracking echocardiography was used to study LV mechanics (LV twisting, LV twisting rate, time to peak twist, LV untwisting rate, time to peak untwist). The exclusion criteria were the presence of coronary artery disease, hypertension, type 2 diabetes.

Results. In the group of patients with EO (+), a significant increase in the level of all studied profibrotic markers was revealed. According to the results of speckle-tracking echocardiography in the EO (+) group, an increase in the LV untwisting rate to -128,31 (-142,0; -118,0) deg/s^{-1} (p=0,002) and an increase in the time to peak untwist to 476,44 (510,0; 411,0) ms compared to the EO (-) group (p=0,03). A weak significant effect of EFT on LV untwisting rate was revealed in the EO (+) group (r=0,24; p=0,04). In addition, a significant relationship was found between the LV untwisting rate and markers of myocardial fibrosis: MMP-3 (r=0,21; p=0,04) and type III collagen (r=0,26; p=0,03).

Conclusion. Thus, the obtained data suggest that patients with EO have signs of preclinical LV diastolic dysfunction, which are characterized by an increase in LV untwisting rate and level of serum profibrotic factors.

Keywords: obesity, left ventricular mechanics, myocardial fibrosis markers.

Relationships and Activities: none.

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Myocardial fibrosis is recognized as a key pathological process in cardiovascular disease development [1, 2]. This is a fundamental process that is observed in heart remodeling and is considered one of the main morphological mechanisms for development of heart failure (CH) with preserved left ventricular ejection fraction (LV EF) (HFpEF) and its progression [3]. Nowadays, an increased interest in the study of LV diastolic dysfunction (DD) is appeared. However, the study of this important pathophysiological aspect is currently complicated by the difficulty of early DD diagnosis. Since LV DD is almost asymptomatic at earliest stages, patients visit a doctor at later stages, when a severe clinical picture is already developing [4]. Traditionally, LV EF is used as the main prognostic indicator of cardiac dysfunction. However, it is becoming increasingly clear that the HF prognosis is not easy to assess only according to LV EF, especially in patients with HFpEF [5]. HFpEF accounts for almost half of HF cases. Concomitant diseases, including obesity, arterial hypertension (AH), and diabetes are key factors in HFpEF onset and progression. Recent evidence suggests that in HFpEF, the degree of myocardial fibrosis is associated with DD degree. In spite of that, the evaluation of fibrosis is not part of everyday clinical practice. First of all, this is due to the difficulties of non-invasive diagnosis of myocardial fibrosis [1]. Classical two-dimensional echocardiography (EchoCG) does not provide information on presence or degree of fibrosis. Available methods of ultrasound diagnostics of LV DD have several algorithms, but there are "blind areas" — combinations of ultrasound signs when LV DD cannot be detected [7-9]. Currently, there is a search for EchoCG methods to detect DD at early stage, since in clinical practice, DD is detected already with sufficiently pronounced fibrosis. These methods include the study of LV mechanics using speckle-tracking EchoCG. In addition, serum markers of myocardial fibrosis are valuable for predicting clinical risk. Extracellular matrix proteins or their cleavage products often enter the systemic circulation and can therefore be measured in serum or plasma. Commonly used fibrosis biomarkers provide insight into collagen production or secretion of non-structural (glyco) proteins that modulate the collagen production itself or its maturation [6].Thus, the search for diagnostic tools for detecting myocardial fibrosis at the initial stages in order to prevent its progression, thereby slowing down the progression of heart failure, is relevant.

Goal of the study: to study the relationship between LV mechanics and the level of myocardial fibrosis serum markers in patients with epicardial obesity (EO).

Material and methods

In this study, from 2016 to 2018, 143 men were enrolled in the Altai Regional Cardiology Dispensary, with an average age of 54,3±8,2 years, who signed an informed consent before being enrolled. The enrollment criteria for patients were the presence of obesity of I-III degree, the average body mass index (BMI) was 33,7±3,3 kg/m^2. The exclusive criteria were the presence of hypertension, coronary atherosclerosis, type 2 diabetes mellitus, as well as the presence of myocardial DD according to transthoracic EchoCG. DD was detected in 33 patients, who were subsequently excluded from the analysis.

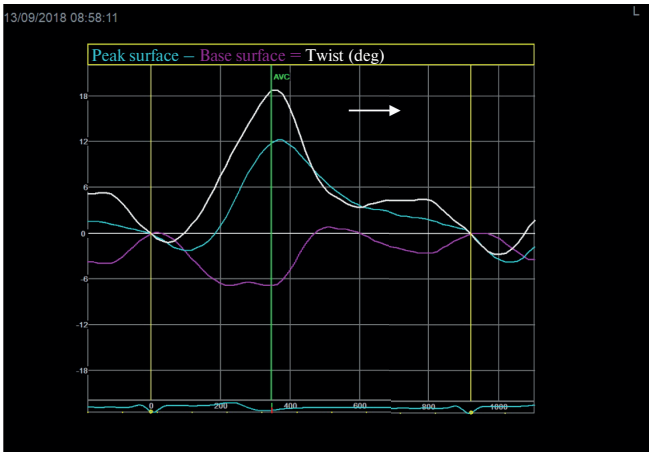


Figure 1. Speckle-tracking EchoCG: LV twist.
Note: the graph shows LV twist in M patient.

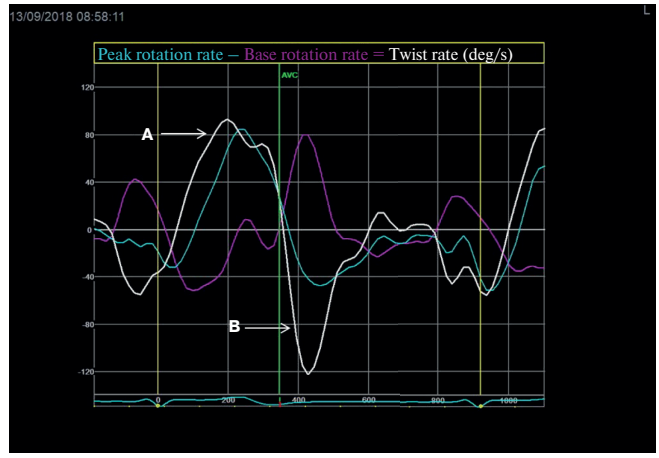


Figure 2. Speckle-tracking EchoCG: LV twisting rate and LV untwisting rate.
Note: the graph below the letter A shows LV twisting rate, under the letter B — LV untwisting rate in M patient.

In all patients, the following laboratory parameters were detected in blood serum using commercially available enzyme immunoassay kits in accordance with the manufacturer's recommendations: levels of type I and III collagen and procollagen I C-terminal propeptide (PICP) were detected using the Cloud-Clone Corp kit (Cloud-Clone Corp., USA); levels of other myocardial fibrosis markers, such as matrix metalloproteinase-3 (MMP-3), transforming growth factor-β (TGF-β), vascular Endothelial growth factor (VEGF-A) was dectected using ebioscience kits (ebioscience, Austria).

The criterion for obesity was BMI ⩾30 kg/m^2 (Society of cardiology of Russian Federation, 2009). BMI was calculated using the formula weight (kg)/ height (m)^2.

All patients underwent EchoCG on the ultrasound system VIVID E95 (GE Healthcare) with matrix sector phased sensor M5Sc (1,5-4,5 MHz). Linear thickness of epicardial adipose tissue (tEAT) as an equivalent of EO was measured in parasternal position along the long LV axis behind free right ventricle wall at the end of systole along the line maximally perpendicular to fibrous ring of aortic valve, which was used as an anatomical landmark in B-mode [10]. The 2016 EACVI recommendations were used to detect LV DD in patients with preserved EF [4]. The rate of LV longitudinal elongation in early diastole (by mitral ring velocity, e'), the ratio of transmitral diastolic flow velocity E to average mitral ring velocity E/e', the left atrium (LA) volume index, and the maximum tricuspid regurgitation rate were

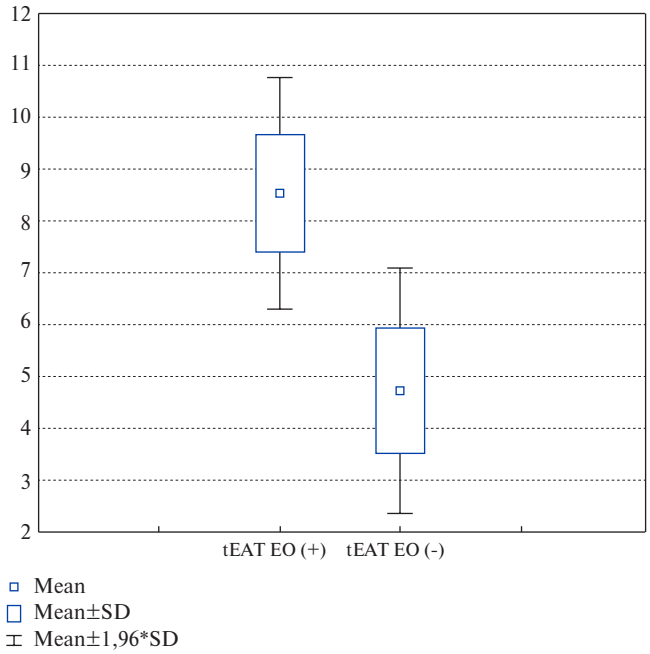


Figure 3. The average values of tEAT in EO (+) and EO (-) groups.
Abbreviations: tEAT — thickness of epicardial adipose tissue, EO — epicardial obesity.

Table 1

Analysis of myocardial fibrosis markers in groups with and without EO

Parameters \ Groups	Group EO (+) (n=70)	Group EO (-) (n=40)	p
MMP-3, ng/ml, (HQ; LQ)	19,47 (24,58; 12,53)	11,16 (13,25; 9,56)	<0,001
Collagen I, pg/ml, M±SD	39958,91±1108,15	25761,30±1885,38	<0,001
Collagen III, pg/ml, M±SD	39821,13±1048,59	28772,25±1090,30	<0,001
TGF-β, ng/ml, M±SD	46,90±1,48	33,62±1,42	<0,001
VEGF-A, pg/ml, M±SD	77,09±1,98	63,74±1,97	<0,001
PICP, pg/ml, M±SD	775,70±17,52	628,07±18,03	<0,001

Note: p — achieved significance level.
Abbreviations: MMP-3 — matrix metalloproteinase-3, EO — epicardial obesity, PICP — procollagen I of C-terminal propeptide, TGF-β — transforming growth factor-β, VEGF-A — vascular endothelial growth factor.

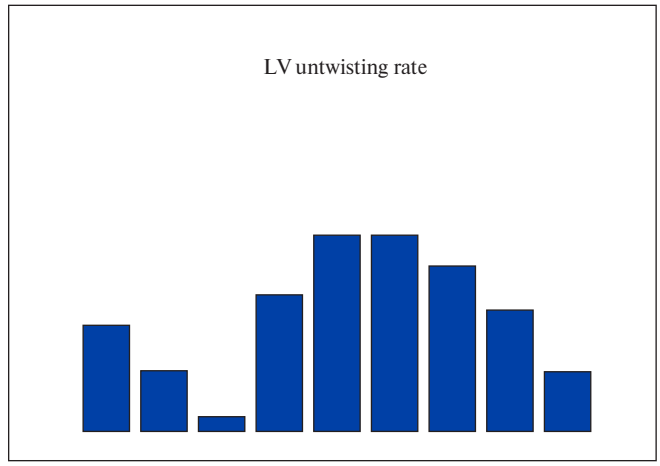
Table 2

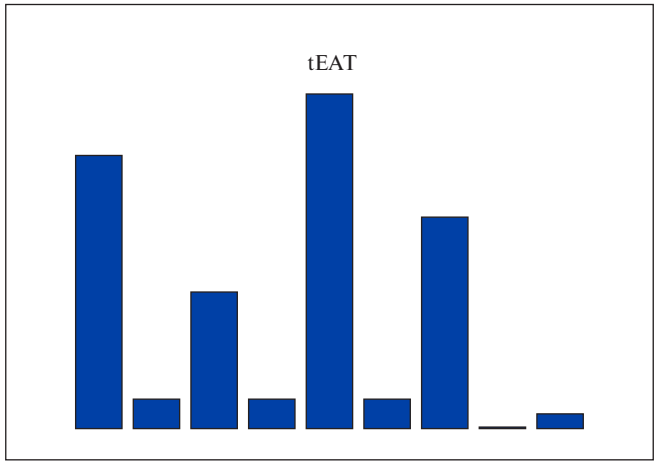
Analysis of EchoCG parameters with and without EO

Parameters \ Groups	Group EO (+) (n=70)	Group EO (-) (n=40)	p
e', cm/s, (HQ; LQ)	0,09 (0,11; 0,09)	0,09 (0,11; 0,09)	0,63
E/e'avg, standard unit, (HQ; LQ)	7,80 (8,90; 6,55)	8,53 (9,70; 7,20)	0,08
Volume index of left atrium, ml/sq^2, (HQ; LQ)	28,39 (31,25; 24,17)	27,82 (30,21; 25,66)	0,55
Maximum speed of tricuspid regurgitation, m/s, (HQ; HQ)	2,78 (2,9; 2,58)	2,67 (2,87; 2,41)	0,13

Note: p — achieved significance level.
Abbreviations: e' — speed of lateral part of mitral valve fibrous ring, E/e'avg — ratio of speed of transmitral diastolic flow to average speed of mitral ring movement, EO — epicardial obesity.

detected [4]. In order to study the LV mechanics, EchoCG was performed in two-dimensional mode according to the standard technique from parasternal access along the short LV axis at the level of mitral valve and apical segments. In the cine loop mode, three cardiac cycles were recorded, and then the LV mechanics were evaluated using ultrasound technology of two-dimensional Speckle Tracking Imaging using an analytical program (EchopacPC, GE Healthcare). The curves obtained at the level of mitral valve and apical segments were used to calculate the LV rotation at the end of systole at the basal (RotMV) and apical (Rotapex) levels, expressed in degrees. Normal apex movement in





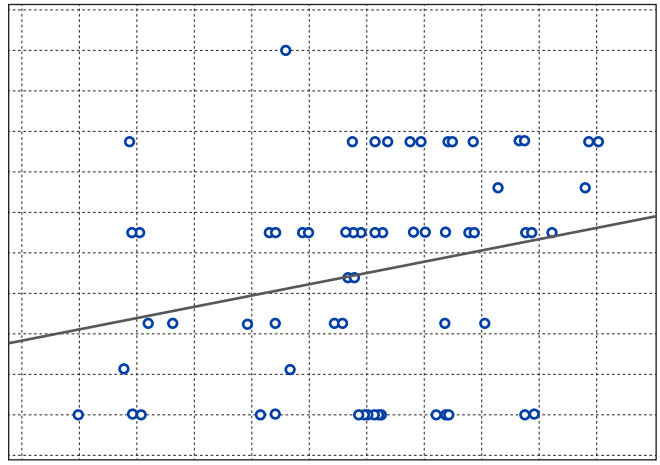


Figure 4. Spearman correlation analysis of LV untwisting rate — tEAT in the group with EO.
Abbreviations: LV — left ventricle, tEAT — thickness of epicardial adipose tissue.

Table 3

Spearman correlation analysis of EchoCG parameters and myocardial fibrosis markers in groups with and without EO

Groups	EO (+) (n=70)				EO (-) (n=40)			
Parameters	e'	E/e'	LA volume index	Maximum TR speed	e'	E/e'	LA volume index	Maximum TR speed
MMP-3, ng/ml	r=-0,10; p=0,39	r=0,09; p=0,48	r=-0,16; p=0,18	r=-0,07; p=0,57	r=0,23; p=0,22	r=-0,10; p=0,58	r=0,05; p=0,79	r=0,10; p=0,59
Collagen I, pg/ml	r=-0,04; p=0,76	r=0,12; p=0,31	r=-0,08; p=0,49	r=-0,06; p=0,64	r=-0,07; p=0,70	r=0,11; p=0,56	r=0,27; p=0,15	r=-0,23; p=0,22
Collagen III, pg/ml	r=-0,01; p=0,94	r=0,08; p=0,49	r=-0,15; p=0,23	r=-0,16; p=0,19	r=-0,14; p=0,44	r=0,15; p=0,41	r=0,25; p=0,17	r=0,32; p=0,08
TGF-β, ng/ml	r=-0,01; p=0,94	r=-0,15; p=0,23	r=-0,23; p=0,05	r=0,12; p=0,31	r=0,25; p=0,17	r=-0,30; p=0,10	r=-0,06; p=0,74	r=-0,15; p=0,41
VEGF-A, pg/ml	r=-0,02; p=0,89	r=-0,09; p=0,42	r=-0,12; p=0,34	r=-0,002; p=0,99	r=-0,15; p=0,42	r=0,16; p=0,40	r=0,31; p=0,09	r=0,34; p=0,06
PICP, pg/ml,	r=0,07; p=0,56	r=0,12; p=0,34	r=-0,03; p=0,79	r=-0,18; p=0,14	r=-0,02; p=0,92	r=-0,04; p=0,82	r=-0,02; p=0,90	r=0,10; p=0,59

Note: p — achieved significance level, r — rank correlation coefficient.
Abbreviations: LA — left atrium, MMP-3 — matrix metalloproteinase-3, TR — tricuspid regurgitation, EO — epicardial obesity, PICP — procollagen I of C-terminal propeptide, TGF-β — transforming growth factor-β, VEGF-A — vascular endothelial growth factor, e' — speed of lateral part of mitral valve fibrous ring, E/e'avg — ratio of speed of transmitral diastolic flow to average speed of mitral ring movement.

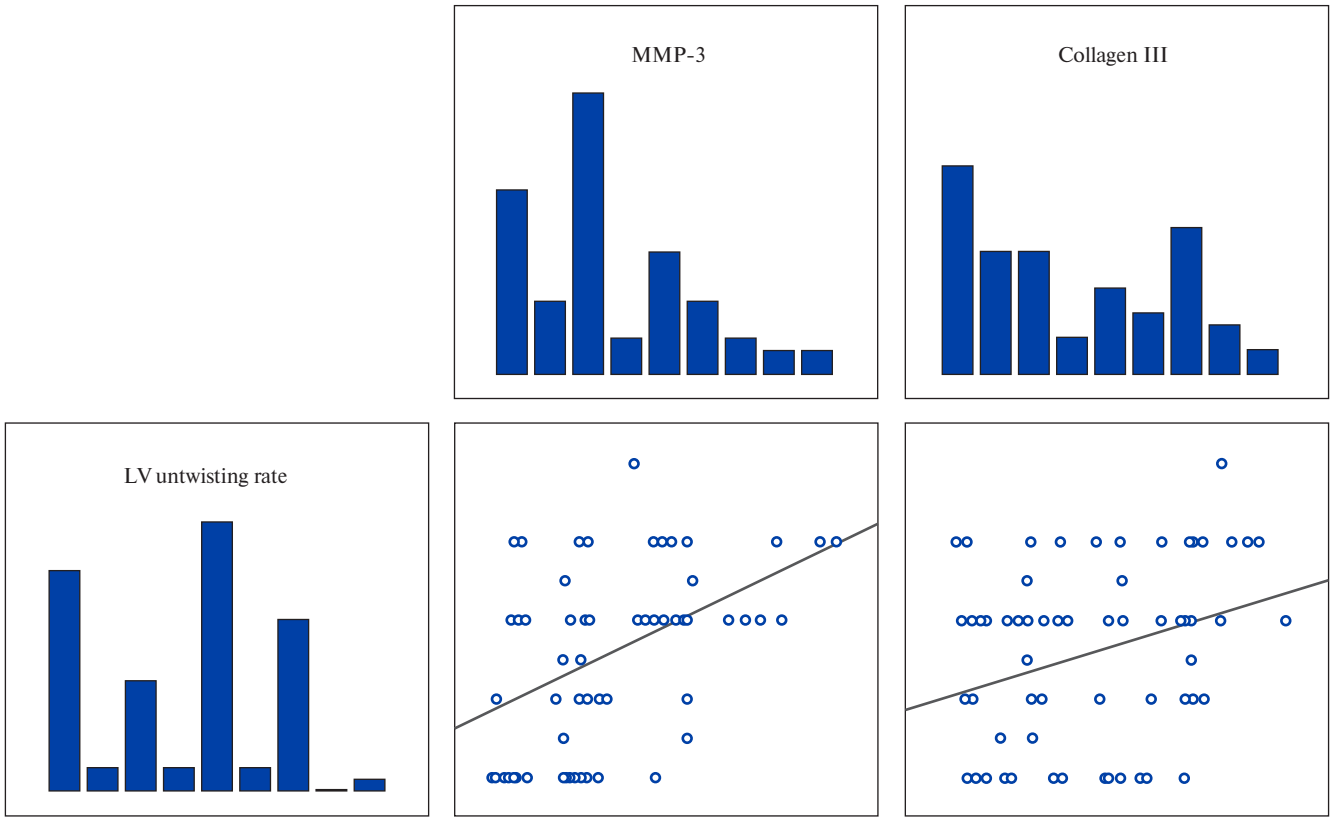


Figure 5. Spearman correlation analysis of LV untwisting rate with markers of myocardial fibrosis in both groups.
Abbreviations: LV — left ventricle, MMP-3 — matrix metalloproteinase-3.

Table 4

Analysis of LV mechanics indicators in both groups

Parameters \ Groups	Group EO (+) (n=70)	Group EO (-) (n=40)	p
Twisting, deg, (HQ; LQ)	19,56 (22,0; 17,36)	15,39 (21,0; 11,8)	0,14
Twisting speed, deg/s^{-1}, (HQ; LQ)	118,70 (124,7; 101,70)	97,25 (117,0; 85,0)	0,16
Time to twist peak, msec, (HQ; LQ)	186,90 (224,0; 148,0)	179,44 (214,0; 131,0)	0,83
Untwisting rate, deg/s^{-1}, (HQ; LQ)	-128,31 (-142,0; -118,0)	-89,68 (-89,0; -78,75)	0,002
Time to untwisting peak, msec, (HQ; LQ)	476,44 (510,0; 411,0)	402,50 (361,0; 415,0)	0,03

Abbreviation: EO — epicardial obesity.

systole implies counterclockwise movement, is plotted on the graph as a curve directed upward from the isoline, and is evaluated as a positive value. Normal basal areas rotation is associated with a clockwise movement, which is shown on the graph as a downward curve and is evaluated in negative values. The resulting LV twist was quantified as the degree-expressed rotation of apex minus the value of rotation at the basal level [11] (Figure 1, 2). The LV twisting rate (deg/s^{-1}), the time to LV twisting peak (as the first positive peak after R wave on electrocardiogram, msec), the untwisting rate (during early diastole, deg/s^{-1}) and the time to LV untwisting peak (as the first negative peak after aortic valve closure, msec) [12].

In order to exclude AH, including masked forms, daily blood pressure monitoring was performed with the device MD-01M (Russia).

To exclude atherosclerotic damage of coronary arteries, patients were given either multispiral computed tomography of coronary arteries or coronary angiography, according to the indications. Multispiral computed tomography of coronary arteries was carried out using a multispiral X-ray computed tomograph from Toshiba (Japan), a 64-slice tomograph with data processing at the workstation VITREA, and the

coronary angiography Integris 3000 by Philips (the Netherlands).

Depending on presence or absence of EO, patients were divided into two groups: EO (+) with tEAT ⩾7 mm (n=70) and EO (-) with a tEAT <7 mm (n=40). EO was considered an increase in tEAT ⩾7 mm, which in clinical trials showed an association with the risk of developing insulin resistance, dyslipidemia, and other metabolic disorders [13]. The selected groups did not differ in age, gender, systolic and diastolic blood pressure, waist circumference and hip circumference, BMI.

Statistical data processing was carried out with the help of the program STATISTICA 10. For each of the continuous quantities with a normal distribution, the mean (M) and standard deviation (SD) are given, for quantities with an abnormal distribution, the median (Me) and the upper and lower quartiles (HQ; LQ) are given. The normal distribution hypothesis was checked using the Shapiro-Wilk test. Statistical description of relationship between the various parameters was carried out by calculating the Spearman rank correlation coefficient. The level of statistical significance was assumed to be $p<0,05$.

Results and discussion

When comparing the average values of tEAT in the selected groups, it was found that in the group EO (+), it was 1,8 times greater than in the group EO (-), and amounted to 8,54 (7,0; 9,0) mm, and in the group EO (-) — 4,74 (4,0; 6,0) mm, $p<0,001$ (Figure 3).

When studying the features of changes in the level of myocardial fibrosis serum markers, a statistically significant increase in the level of all the studied markers was revealed in the group EO (+) compared to the group EO (-) (Table 1).

When analyzing the echocardiography data, it was revealed that the selected groups did not have statistically significant differences in such parameters as e', E/e', LA volume index, and maximum tricuspid regurgitation rate (Table 2).

A correlation analysis was performed between the EchoCG parameters (e', E/e', LA volume index, maximum tricuspid regurgitation rate) and the level of myocardial fibrosis markers. Correlations between the studied parameters in both groups were not revealed (Table 3).

When assessing the average parameters of LV mechanics in the group EO (+), an increase in the LV untwisting rate by 1,4 times and an increase in the time to LV untwisting peak by 1,2 times were revealed (Table 4).

The correlation analysis revealed that in the group EO (+), there is a weak statistically significant effect of tEAT on the LV untwisting rate ($r=0,24$; $p=0,04$), while in the EO (-) group, there is no such effect (Figure 4).

When assessing the relationship between LV mechanics indicators associated with LV diastolic function (LV untwisting rate and time to peak of LV untwisting) and serum markers of myocardial fibrosis, the Spearman correlation analysis was performed and positive statistically significant relationship between LV untwisting rate and matrix metalloproteinase-3 (MMP-3) ($r=0,21$; $p=0,04$), as well as with type III collagen ($r=0,26$; $p=0,03$) in the group EO (+), no relationships were found with other markers of myocardial fibrosis. No such relationships were observed in the group EO (-) (Figure 5).

Violation of LV DD is formed in the early stages of course of many diseases connected to cardiovascular system. The influence of obesity on development and progression of lipotoxic myocardial fibrosis and DD has been proven. Considering the fact that there are currently no non-invasive methods aimed at early detection of myocardial fibrosis, the morphological basis of DD, with the subsequent HF development, we made an attempt to assess the levels of pro-fibrotic markers in patients with and without EO, to assess the parameters of LV mechanics that characterize LV diastolic function, and to identify the relationship between these parameters. In our study, it was shown that in patients with EO, the level of all studied serum markers of myocardial fibrosis (MMP-3, collagen I, collagen III, TGF-β, VEGF-A, PICP) increases. When assessing the parameters of LV mechanics, which change at the earliest stages of myocardial damage, it was found that the LV untwisting rate and the time to LV untwisting peak increased, while the EchoCG indicators recommended for LV DD detection were not changed. Our data is consistent with the literature data. Thus, in a study conducted by Ahmed MK, et al., aimed at studying the mechanics of LV in DD, it was shown that the LV untwisting rate and the time to LV untwisting peak in the early DD stages (with impaired relaxation) increase, then these indicators decrease in the course of HF progression, and these indicators normalize or decrease in patients with an E/A value >1,5 [14].

Conclusion

Thus, the data obtained by us showed that the enrolled patients with EO have signs of preclinical LV DD. The relationship between the LV untwisting rate and MMP-3, type III collagen, was revealed, while there is no relationship between these markers and traditional EchoCG criteria for LV DD. The obtained data suggest that the determination of the

level of myocardial fibrosis serum markers, as well as the determination of the LV untwisting rate and the time to LV untwisting peak using speckle-tracking EchoCG, which reflect the mechanical DD aspect, can be used as additional markers for DD detection at the preclinical stage.

Relationships and Activities: none.

References

1. Hassan S, Barrett CJ, Crossman DJ. Imaging tools for assessment of myocardial fibrosis in humans: the need for greater detail. Biophys Rev. 2020;12(4):969-87. doi:10.1007/s12551-020-00738-w.
2. Chumakova GA, Veselovskaya NG. Clinical significance of visceral obesity. Moscow: GEOTAR-Media 2016. p 155 (In Russ.) ISBN: 978-5-9704-3988-3.
3. Piek A, de Boer RA, Silljé HH. The fibrosis-cell death axis in heart failure. Heart Fail Rev. 2016;21:199-211. doi:10.1007/s10741-016-9536-9.
4. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J. Am. Soc. Echocardiogr. 2016;29(4):277-314. doi:10.1016/j.echo.2016.01.011.
5. Whalley GA. Surrogate Survival: Battle Between Left Ventricular Ejection Fraction and Global Longitudinal Strain. JACC: Cardiovascular Imaging. 2018;11(11):1580-2. doi:10.1016/j.jcmg.2017.11.003.
6. de Boer RA, de Keulenaer G, Bauersachs J, et al. Towards better definition, quantification and treatment of fibrosis in heart failure. A scientific roadmap by the Committee of Translational Research of the Heart Failure Association (HFA) of the European Society of Cardiology. Eur J Heart Fail. 2019;21(3):272-85. doi:10.1002/ejhf.1406.
7. van Dalen BM, Strachinaru M, van der Swaluw J, et al. A simple, fast and reproducible echocardiographic approach to grade left ventricular diastolic function. Int. J. Cardiovasc. Imaging. 2016;2(5):743-52. doi:10.1007/s10554-015-0832-6.
8. Otto CM. The practice of clinical echocardiography. Philadelphia Pennsylvania: Elsevier, 5th Edition, 2016. p. 1024.
9. Zherko OM. Echocardiographic criteria of diastolic dysfunction of the left ventricle and their diagnostic reliability. Vestnik VGMU. 2020;19(3):87-95. (In Russ.) doi:10.22263.2312-4156.2020.3.87.
10. Iacobellis G, Willens HJ. Echocardiographic Epicardial Fat: A Review of Research and Clinical Applications. JASE. 2009;22(12):1311-9. doi:10.1016/j.echo.2009.10.013.
11. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J. Am. Soc. Echocardiogr. 2016;29(4):277-314. doi:10.1016/j.echo.2016.01.011.
12. Pavlyukova EN, Kuzhel DA. Left ventricle torsion in an idiopathic his left bundle branch block. Russ J Cardiol. 2017;(7):121-4. (In Russ.) doi:10.15829/1560-4071-2017-7-121-124.
13. Ahmed MK, Soliman MA, Mena MB, et al. Relation of Diastolic Dysfunction to the LV Twist and Untwist Dynamic: Speckle Tracking Imaging Study. Int J Cardiovasc Res. 2019;7:5. doi:10.4172/2324-8602.1000362.
14. Veselovskaya NG, Chumakova GA, Ott AV, et al. Alternative method of visceral obesity assessment in the diagnostics of metabolic syndrome. Russian Journal of Cardiology. 2014;(3):82-6. (In Russ.) doi:10.15829/1560-4071-2014-3-82-86.
15. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Europ. Heart J. 2016;37(27):2129-200. doi:10.1093/eurheartj/ehw128.