УДК 616.12-008.46:612.172
https://doi.org/10.20538/1682-0363-2022-4-88-97

Heart failure with preserved ejection fraction: the role of microvascular dysfunction

Kopeva K.V., Mochula A.V., Maltseva A.N., Grakova E.V., Shipulin V.V., Gusakova A.M., Zavadovsky K.V.

Cardiology Research Institute, Tomsk National Research Medical Center (NRMC), Russian Academy of Sciences
111a, Kievskaya Str., Tomsk, 634012, Russian Federation

ABSTRACT

Aim. To evaluate the relationship between coronary microvascular dysfunction (CMD), biomarkers of cardiac fibrosis and cardiac remodeling (soluble ST2 (sST2), fibroblast growth factor-23 (FGF-23), matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1), and NT-proBNP), parameters of diastolic dysfunction (DD), and the presence of heart failure with preserved ejection fraction (HFpEF) in symptomatic patients.

Materials and methods. Study participants were 59 patients with non-obstructive coronary artery disease (CAD) and preserved left ventricular ejection fraction (LVEF) of 62 (56; 67) %. Non-obstructive CAD was verified by coronary computed tomography angiography. Stress- and rest-myocardial blood flow (MBF) and coronary flow reserve (CFR) parameters were evaluated by CZT SPECT. Serum levels of cardiac biomarkers were measured by the enzyme immunoassay. Two-dimensional transthoracic echocardiography was used to assess DD parameters.

Results. Decreased CFR was defined as CFR ≤ 2. Therefore, CMD was defined as the presence of decreased CFR in the absence of flow-limiting CAD. Distribution of patients was performed by CFR values: group 1 included patients with preserved CFR (>2, n = 35), and group 2 encompassed patients with decreased CFR (≤2, n = 24). In 87.5% of cases, patients with CMD were diagnosed with HFpEF, whereas in patients with preserved CFR, heart failure was diagnosed only in 51.4% of cases ($p < 0.0001$). CFR values were correlated with the left atrial volume ($r = –0.527$; $p = 0.001$), E / A ratio ($r = –0.321$, $p = 0.012$), and E / e' ($r = –0.307$; $p = 0.021$). Following the ROC analysis, the levels of sST2 ≥ 31.304 ng / ml (AUC = 0.730; p = 0.004) and NT-proBNP ≥ 0.034 pg / ml (AUC = 0.815; $p = 0.034$) were identified as cut-off values for the presence of CMD in patients with non-obstructive CAD.

Conclusion. The obtained data suggest that CMD may play an essential role in HFpEF. The values of CFR were correlated with DD parameters, and decreased CFR was associated with overexpression of biomarkers of cardiac fibrosis and cardiac remodeling. Serum levels of sST2 and NT-proBNP were identified as cut-off values for the presence of CMD in patients with non-obstructive CAD.

Keywords: heart failure, preserved left ventricular ejection fraction, diastolic dysfunction, coronary flow reserve, myocardial blood flow, microvascular dysfunction

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The study was supported by the grant awarded by the President of the Russian Federation MK-4257.2022.3.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Cardiology Research Institute of Tomsk NRMC (Protocol No. 204 of 18.11.2020).

For citation: Kopeva K.V., Mochula A.V., Maltseva A.N., Grakova E.V., Shipulin V.V., Gusakova A.M., Zavadovsky K.V. Heart failure with preserved ejection fraction: the role of microvascular dysfunction. *Bulletin of Siberian Medicine.* 2022;21(4):88–97. https://doi.org/10.20538/1682-0363-2022-4-88-97.

✉ *Kopeva Kristina V.,* Kristin-kop@inbox.ru

Сердечная недостаточность с сохраненной фракцией выброса: роль микроваскулярной дисфункции

Копьева К.В., Мочула А.В., Мальцева А.Н., Гракова Е.В., Шипулин В.В., Гусакова А.М., Завадовский К.В.

Научно-исследовательский институт (НИИ) кардиологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук
Россия, 634012, Томск, ул. Киевская, 111а

РЕЗЮМЕ

Цель. Оценить взаимосвязь между коронарной микроваскулярной дисфункцией (КМД), биомаркерами фиброза и миокардиального ремоделирования (растворимый ST2 (sST2) и фактор роста фибробластов 23 (FGF-23), матриксная металлопротеиназа-9 (ММП-9), тканевой ингибитор металлопротеиназ-1 (ТИМП-1), NT-proBNP), параметрами диастолической дисфункции (ДД) и наличием сердечной недостаточности с сохраненной фракцией (СНсФВ) у симптоматичных пациентов.

Материалы и методы. В исследование включены 59 пациентов с необструктивным поражением коронарных артерий (КА) и сохраненной фракцией выброса левого желудочка (ФВ ЛЖ) 62 (56; 67)%. Необструктивное поражение КА было подтверждено компьютерной коронарной ангиографией. С помощью динамической CZT-SRECT оценивали параметры миокардиального кровотока в состоянии покоя (rest-MBF) и стресса (stress-MFR) и резерва коронарного кровотока (CFR). Сывороточные уровни сердечных биомаркеров измеряли с помощью иммуноферментного анализа. Всем пациентам проводилась двухмерная трансторакальная эхокардиография для оценки параметров ДД.

Результаты. Сниженный CFR определяли как CFR ≤2. Таким образом, КМД диагностировали на основании сниженного CFR при отсутствии окклюзирующего поражения КА. Распределение пациентов проводилось по значениям CFR: группа 1 включала больных с сохраненным CFR (>2, n = 35), группа 2 – со сниженным CFR (≤2, n = 24). В 87,5% случаев у больных с КМД была диагностирована СНсФВ, тогда как у больных без КМД – только в 51,4% (p < 0,0001). Значения CFR коррелировали с объемом левого предсердия (r = –0,527; p = 0,001), отношением E/A (r = –0,321; p = 0,012) и E/e' (r = –0,307; p = 0,021). На основании ROC-анализа уровни sST2 ≥ 31,304 нг/мл (AUC = 0,730; p = 0,004) и NT-proBNP ≥ 0,034 пг/мл (AUC = 0,815; p = 0,034) были определены как пороговые значения для диагностики КМД у пациентов с необструктивным поражением КА.

Заключение. КМД может играть важную роль в патогенезе развития СНсФВ. Значения CFR коррелировали с параметрами ДД, а снижение CFR было связано с гиперэкспрессией сердечных биомаркеров фиброза и ремоделирования. Уровни sST2 и NT-proBNP могут использоваться в качестве маркеров неинвазивной диагностики КМД.

Ключевые слова: сердечная недостаточность, сохраненная фракция выброса левого желудочка, диастолическая дисфункция, коронарный резерв, миокардиальный кровоток, микрососудистая дисфункция

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование поддержано грантом президента Российской Федерации (МК-4257.2022.3).

Соответствие принципам этики. Информированное письменное согласие было получено от всех пациентов до их включения в данное исследование. Исследование одобрено локальным комитетом по этике НИИ кардиологии Томского НИМЦ (протокол № 204 от 18.11.2020).

Для цитирования: Копьева К.В., Мочула А.В., Мальцева А.Н., Гракова Е.В., Шипулин В.В., Гусакова А.М., Завадовский К.В. Сердечная недостаточность с сохраненной фракцией выброса: роль микроваскулярной дисфункции. *Бюллетень сибирской медицины.* 2022;21(4):88–97. https://doi.org/10.20538/1682-0363-2022-4-88-97.

INTRODUCTION

Despite growing prevalence worldwide, heart failure with preserved ejection fraction (HFpEF) remains a poorly understood clinical syndrome [1, 2]. At the same time, a lack of clear understanding of its pathophysiology results in a lack of effective targeted therapy [3, 4]. Recent studies have implicated that coronary microvascular dysfunction (CMD) may be one of the possible causes of development and progression of HFpEF [5, 6].

Coronary flow reserve (CFR), quantified as the ratio of hyperemic myocardial blood flow to resting myocardial blood flow, reflects functional ischemia in large and small vessels. In the absence of obstructive coronary artery disease (CAD), it is a marker of CMD [7]. A new class of gamma cameras equipped with semiconductor cadmium – zinc – telluride (CZT) detectors has recently made it possible to measure CFR by noninvasive dynamic SPECT imaging [8, 9]. This method has been sufficiently tested and validated and may be a more accessible technique for visualization of changes in the coronary microcirculation [10] in addition to a comprehensive clinical assessment and traditional tests for assessing stress-induced ischemia [11].

The potential mechanisms of CMD appear to be heterogeneous, including impaired endothelial function, systemic inflammation, mitochondrial dysfunction, oxidative stress, etc. [12–16]. Moreover, all these processes cause adhesion and infiltration of monocytes and stimulation of integrated macrophages that promote myofibroblast differentiation and collagen secretion leading to fibrosis and cardiac remodeling [10, 11, 13–15]. Thus, CMD may play an important role in elevated left ventricular (LV) filling pressure, the development of diastolic dysfunction (DD), and the pathophysiology of HFpEF in general [7, 16].

The aim of the study was to evaluate the relationship between CMD, biomarkers of fibrosis and cardiac remodeling (soluble ST2 (sST2), fibroblast growth factor-23 (FGF-23), matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1), NT-proBNP), diastolic dysfunction (DD) parameters, and the presence of HFpEF in symptomatic patients.

MATERIALS AND METHODS

The study was performed in accordance with the Declaration of Helsinki and was approved by the local Ethics Committee at Cardiology Research Institute, Tomsk NRMC (protocol No.204 of 18.11.2020). An informed written consent was obtained from all patients prior to their enrollment in the study.

Study population. From December 2020 to January 2022, a total of 59 patients (39 men, average age of 65.0 [58.0; 69.0] years) were enrolled in the study. All patients did not receive optimal medical treatment before the enrollment. Inclusion criteria: 1) non-obstructive (< 50%) coronary artery disease (CAD); 2) documented left ventricular ejection fraction (LVEF) ≥ 50% measured by echocardiography; 3) LVDD / elevated left ventricular filling pressure (LVFP) based on echocardiography; 4) sinus rhythm; 5) a signed informed consent to participate in the study.

Exclusion criteria were the following: 1) myocardial infarction in the medical history; 2) planned coronary revascularization and / or previous revascularization of the coronary artery (CA); 3) systolic blood pressure > 160 mm Hg); 4) symptomatic hypotension with the mean systolic blood pressure < 90 mm Hg; 5) second- or third-degree atrioventricular block, sick sinus syndrome; 6) persistent or chronic atrial fibrillation and / or flutter; 7) valvular insufficiency or stenosis of ≥ 2 degree; 8) hypertrophic and dilated cardiomyopathy; 9) previous pulmonary embolism with pulmonary hypertension of ≥ 45 mm Hg; 10) severe bronchial asthma and / or chronic obstructive pulmonary disease; 11) pathology of the thyroid gland; 12) glomerular filtration rate (CKD-EPI) of < 30 ml / min / m^2; 13) class 3 hepatic insufficiency according to Child-Pugh classification; 14) acute and chronic inflammatory heart diseases; 15) hemoglobin level of < 100 g / dl; 16) stroke or transient ischemic attack within 90 days prior to enrollment; 17) obesity (body mass index (BMI) > 35 kg / m^2); 18) life-threatening uncontrolled arrhythmias.

Echocardiography. Philips Affiniti 70 ultrasound scanner was used to perform two-dimensional transthoracic echocardiography. All studies were performed by one highly qualified specialist. Evaluation of LVDD was based on the following indices: E wave, E/A ratio, septal e′, average E/e′ ratio, indexed left atrial volume, and peak tricuspid regurgitation velocity [17].

Coronary computed tomography angiography and dynamic SPECT. Dynamic CZT SPECT and coronary computed tomography angiography (CCTA) were performed using a hybrid system (GE Discovery NM/CT 570C; GE Healthcare, USA) equipped with a dedicated cardiac CZT gamma camera and a 64-slice CT scanner.

Dynamic SPECT. Patient preparation, study protocol, as well as acquisition and analysis of static and dynamic scintigraphic data were described in the previous articles [9, 10]. It is important to note that patients were instructed to stop taking beta-blockers, nitrates, calcium channel blockers, caffeine, and methylxanthine-containing substances for at least 24 hours before the procedure. All studies were performed in the morning, on an empty stomach, against the background of a sinus heart rhythm [18]. A two-day rest – stress protocol was performed using the radiopharmaceutical 99mTc-methoxy-isobutyl-isonitrile (99mTc-MIBI), which was administered intravenously at a bolus dose of 260–444 MBq. Before the first dynamic study, a low-dose CT scan (tube voltage 100 kV, tube current 20 mA, rotation time 0.8 s, helical pitch 0.969 : 1, slice thickness 5 mm) had been performed to assess the heart position.

The pharmacological stress test was performed according to a standard 4-minute protocol [18]. Adenosine triphosphate (ATP) was used as a pharmacological stress agent, which was administered intravenously using an infusion pump at a dose of 160 μg / kg / min for 4 min. During the stress test, after 2 minutes of intravenous infusion of ATP, a dose of 99mTc-Sestamibi (3 MBq·kg-1) was injected. Dynamic data acquisition was started 610 seconds before the radiotracer injection. The infusion of ATP continued for additional 2 minutes.

To correct attenuation, low-dose CT of the chest was performed. All studies were performed on the Discovery NM/CT 570c hybrid computed tomography scanner (GE Healthcare, Milwaukee, WI, USA) equipped with a gamma camera with highly sensitive CZT detectors. The total effective radiation exposure of the study (rest / pharmacological stress test) was ~6.25 mSv.

The resulting scintigraphic images were processed on the specialized Xeleris II workstation (GE Healthcare, Haifa, Israel). Myocardial perfusion, myocardial blood flow (MBF), and coronary flow reserve (CFR)were assessed using specialized software Corridor 4DM SPECT and 4DM Reserve v.2015 (INVIA, Ann Arbor, MI, USA). The quantitative characteristics were processed using the Net Retention model with attenuation correction [19].

According to myocardial perfusion SPECT data, standard semi-quantitative indices of impaired myocardial perfusion were determined: Summed Stress Score (SSS) – the sum of scores during stress, Summed Rest Score (SRS) – the sum of scores at rest, Summed Difference Score (SDS) – the difference between exercise and rest, as well as quantitative parameters: stress-MBF – myocardial blood flow during stress, rest-MBF – myocardial blood flow at rest, and CFR.

Coronary computed tomography angiography. Preparation for CCTA was carried out according to the standard protocol and included beta-blockers and prednisolone, avoiding caffeinated drinks or food, and excluding the use of glucophage (metformin), viagra, etc., and pain medications (advil or motrin). Besides, patients were instructed about contraindications of the procedure related to allergies, pregnancy, and kidney disease. Heart rate and blood pressure were evaluated before each scan. All patients received 0.5 mg of sublingual nitroglycerin tablets.

For contrast-enhanced scans, 70–90 ml of a non-ionic contrast agent (iopamidol 370 mg, Bracco Diagnostics, Italy) was injected intravenously through an 18G antecubital catheter at a flow rate of 5–5.5 ml / s followed by 60 ml of 0.9% NaCl.

In patients with the heart rate ≥ 55 bpm, a retrospective electrocardiogram (ECG)-gated helical scan was acquired, and in those with the heart rate < 55, a prospective ECG-gated protocol was used. The recording parameters were the following: tube voltage of 120 kV, tube current of 300–600 mA using ECG modulation with maximum tube current of 40–80% between phases, and minimum tube current in the remaining phases.

Axial images, curved multiplanar and cross-section reformations, and thin-slab maximum intensity projections were used for dataset analysis. All studies were analysed on the hybrid CT scanner (Advantage Workstation 4.6, GE Healthcare, USA).

In the case of retrospective CCTA scans, images were reconstructed at 75% of the cardiac cycle with a slice thickness of 0.625 mm. In cases of heart rate artefacts, other reconstruction windows were used (from 10% to 90% of the R-R cycle). According to modified American Heart Association criteria, CAs were subdivided into 16 segments [20, 21].

Blood sampling and biochemical analysis. Blood samples were obtained by venipuncture. Adequate samples were centrifuged, serum was separated and stored at –24 °C with a single freeze – thaw cycle. Serum levels of sST2, NT-proBNP, FGF-23, MMP-9, and TIMP-1 were analyzed from the same blood sample by the enzyme immunoassay (NT-proBNP, FGF-23, and TIMP-1, Biomedica, Austria; Presage® ST2 Assay, Critical Diagnostics, San Diego, CA, USA; MMP-9; eBioscience, USA).

Statistical analysis. Statistical processing of the results was performed using Statistica 10.0 software package R, version 2. The data were presented as the median and the interquartile range *Me* (Q_{25}; Q_{75}). To test statistical hypotheses for quantitative variables, the Mann – Whitney test was used when comparing two independent groups. When analyzing qualitative variables, contingency tables were analyzed using the Pearson's $\chi 2$ test. If there were cells with an expected frequency less than 5, then a two-tailed Fisher's exact test or Yates' correction (for 2 × 2 tables) was applied. To search for relationships between the variables, the correlation analysis was used with the calculation of the Spearman's rank correlation coefficients. The cut off scores for the diagnosis of CMD were determined by the ROC-analysis. The critical significance level of the *p*-value was taken equal to 0.05.

RESULTS

Impaired CFR was defined as CFR ≤ 2. Thus, CMD was defined as the presence of impaired CFR in the absence of flow-limiting CAD. Patients were distributed according to CFR values: group 1 included patients with preserved CFR (> 2, *n* = 35), group 2 included patients with impaired CFR (CFR ≤ 2, *n* = 24). HFpEF was diagnosed according to 2021 ESC guidelines for the diagnosis and treatment of acute and chronic HF [22]. The baseline demographic and clinical characteristics of patients did not differ (Table 1). However, in patients with CMD, HFpEF was revealed in 87.5%, while in patients without CMD, it was diagnosed only in 51.4% ($p < 0.0001$). Echocardiography parameters did not differ significantly between the groups (Table 2).

In patients with CMD, CFR values were lower by 47.8% ($p < 0.0001$) than in patients without CMD (1.41 [1.23; 1.55] vs. 2.6 [2.49; 3.38], respectively). In group 1, rest-MBF was 0.74 (0.56; 0.93) ml / min / g, while in group 2, it was 0.48 (0.37; 0.67) ml / min / g ($p = 0.045$). In group 1, stress-MBF was 1.06 (0.91; 1.24) ml / min / g, and in group 2 it was 1.59 (1.19; 1.74) ml / min / g($p = 0.012$). The remaining indices did not differ significantly (Table 3).

Table 1

Clinical and demographic characteristics of patients, *Me* (Q_{25}; Q_{75})

Parameter	Patients with CMD, *n* = 24	Patients without CMD, *n* = 35	*p* value
Age, years	60 (52; 66)	62 (59; 67.5)	0.451
Sex / male, *n* (%)	14 (58.3)	23 (65.7)	0.767
BMI, kg / m^2	29.55 (27.1; 30.7)	31.2 (28.0; 33.41)	0.180
Hypertension, *n* (%)	20 (83.3)	32 (91.4)	0.812
Diabetes mellitus, *n* (%)	6 (25.0)	10 (28.6)	0.761
COPD, *n* (%)	3 (12.5)	5 (14.3)	0.824
Current smoker, *n* (%)	6 (25.0)	9 (25.8)	0.998
Heart failure, *n* (%)	21 (87.5)	18 (51.5)	< 0.0001
eGFR, ml / min / 1.73 m^2	73.5 (59; 81)	69 (65; 79)	0.775
Total cholesterol, mmol / l	4.15 (3.2; 5.98)	4.4 (3.6; 5.4)	0.874
LDL-cholesterol, mmol / l	1.79 (1.3; 3.34)	2.6 (1.8; 3.42)	0.606
HDL-cholesterol, mmol / l	1.36 (1.29; 1.78)	1.23 (1.06; 1.3)	0.239
Triglycerides, mmol / l	1.5 (1.14; 2.23)	1.6 (1.25; 2.2)	0.815
Hemoglobin, g / dl	152 (144; 159)	143 (137; 153.5)	0.121
Potassium, mmol / l	4.3 (4.0; 5.2)	4.2 (3.9; 5.1)	0.981
HbA1c, %	5.8 (5.5; 7.6)	5.6 (5.3; 7.5)	0.091
CRP, g / l	4.1 (3.6; 4.7)	5.2 (2.7; 10.1)	0.998
Fibrinogen, g / l	3.3 (2.9; 3.4)	3.2 (2.7; 3.4)	0.934
sST2, ng / ml	31.03 (27.03; 35.5)	25.0 (21.45; 31.15)	< 0.001
NT-proBNP, pg / ml	318.0 (169.7; 2,106.2)	196.8 (68.1; 510.4)	0.045
MMP-9, ng / ml	1538 (945.4; 1982)	1183 (720.9; 1725)	0.023
TIMP-1, ng / ml	230.2 (107.38; 285.8)	160.78 (58.66; 213.2)	0.012
FGF-23, ng / ml	0.683 (0.383; 0.999)	0.649 (0.5; 0.965)	0.565

Note: HbA1c – glycated hemoglobin; FGF-23 – fibroblast growth factor-23; LDL-cholesterol – low-density lipoprotein cholesterol; HDL-cholesterol – high-density lipoprotein cholesterol; CRP – C-reactive protein; eGFR – estimated glomerular filtration rate (CKD-EPI); TIMP-1 – tissue inhibitor of metalloproteinase-1; COPD – chronic obstructive pulmonary disease.

Table 2

Echocardiography parameters, *Me* (Q_{25}; Q_{75})			
Parameter	Patients with CMD, *n* = 24	Patients without CMD, *n* = 35	*p*-value
Left ventricular ejection fraction, %	65 (63; 66)	65 (64; 67)	0.531
End-systolic diameter, mm	32 (30; 33)	32 (31; 33)	0.886
End-diastolic diameter, mm	50.5 (48;51)	50.5 (49; 51)	0.752
LVMI, g / m^2	91 (88; 95)	84 (79; 90)	0.159
Interventricular septum, mm	10.2 (10; 11)	10.5 (10.5; 11)	0.371
Left ventricular posterior wall, mm	10 (10; 11)	10 (9.5; 10)	0.154
E / A ratio	0.98 (0.73; 1.38)	1.02 (0.86; 1.29)	0.829
Septal e′	5.89 (4.8; 6.45)	5.66 (5.35; 6.25)	0.949
PTRV, m / s	2.89 (2.8; 3.11)	2.91 (2.87; 2.99)	0.852
E / e’	14.75 (13.5; 15.1)	14 (13.3; 14)	0.181
LAVI	33 (29; 37)	32 (29; 33)	0.284
DD type 1, *n* (%)	19 (79.2)	26 (74.3)	0.761
DD type 2, *n* (%)	5 (20.8)	25.7	0.817

Note: DD – diastolic dysfunction; LAVI – left atrial volume index; LVMI – left ventricular mass index; PTRV – peak tricuspid regurgitation velocity.

Table 3

Dynamic SPECT parameters and standard semi-quantitative indices of impaired myocardial perfusion, *Me* (Q_{25}; Q_{75})			
Parameter	Patients with CMD, *n* = 24	Patients without CMD, *n* = 35	*p*-value
Dynamic SPECT indices			
Stress-MBF, ml / min / g	1.06 (0.91; 1.24)	1.59 (1.19; 1.74)	0.012
Rest-MBF, ml / min / g	0.74 (0.56; 0.93)	0.48 (0.37;0.67)	0.045
CFR	1.41 (1.23; 1.55)	2.6 (2.49; 3.38)	<0.0001
Standard semi-quantitative indices of impaired myocardial perfusion			
SSS	2.0 (1.0; 4.0)	2.0 (0; 4.0)	0.566
SRS	0 (0; 1)	0 (0; 1)	0.926
SDS	0.5 (0; 3.0)	2 (0; 3.0)	0.364
Standard semi-quantitative indices of myocardial dysfunction			
Stress ESV, ml	37.0 (30.0; 46.0)	33.5 (25.5; 40.0)	0.158
Stress EDV, ml	115.5 (97.0; 123.0)	106.5 (99.0; 122.5)	0.404
Stress EF, %	68.0 (61.0; 74.0)	70.0 (66.0; 73.5)	0.244
Rest ESV, ml	32.0 (28.0; 41.0)	32.5 (24.5; 36.0)	0.364
Rest EDV, ml	108.5 (100; 117)	102.5 (89.5;121.5)	0.250
Rest EF, %	70.5 (62.0; 72.0)	69.5 (65.5; 72.5)	0.698

Note: CFR – coronary flow reserve; stress-MBF – myocardial blood flow during stress; rest-MBF – myocardial blood flow at rest; SSS – summed stress score; SRS – summed rest scores; SDS – summed difference score as the difference between SSS and SRS; ESV – end-systolic volume; EDV – end-diastolic volume; EF – ejection fraction.

CFR values were correlated with the left atrial volume (r = –0.527; p = 0.001), E / A ratio (r = –0.321, p = 0.012), and E / e’ (r = –0.307; p = 0.021), as well as with the levels of NT-proBNP (r = –0.290; p = 0.04) and sST2 (r = –0.330; p = 0.012).

The levels of NT-proBNP were higher in group 1 by 36.4% (p = 0.045) than in group 2 (318.0 [169.7; 2,106.2] and 196.8 [68.1; 510.4] pg / ml, respectively). The sST2 levels were higher in patients with impaired CFR by 19.4% (p > 0.001) than in patients with preserved CFR (31.03 [27.03; 35.5] and 25.0 [21.45; 31.15] ng / ml, respectively). The serum levels of MMP-9 in group 1 were 1,538 (945.4; 1,982) pg / ml, and in group 2 they were 1,183 (720.9; 1,725) ng /ml (p = 0.023). The levels of TIMP-1 were higher by 30.1% (p = 0.012) in group 1 than in group 2 (230.2 [107.38; 285.8] and 160.78 [58.66; 213.2] ng / ml, respectively). The serum concentration of FGF-23 did not differ between the groups.

Following the ROC-analysis, the levels of sST2 ≥ 31.304 ng / ml (sensitivity 55.0%, specificity 90.3%; area under the curve (AUC) = 0.730; p = 0.004) (Fig. 1) and NT-proBNP ≥ 977.2 pg / ml (sensitivity 64.9%, specificity 84.6%; AUC = 0.815; p = 0.034) were identified as cut-off values for diagnosing CMD

in patients with non-obstructive CAD (Fig. 2). When comparing the ROC-curves of sST2 and NT-proBNP, no significant differences in the cut-off values for the presence of CMD were revealed (Fig. 3).

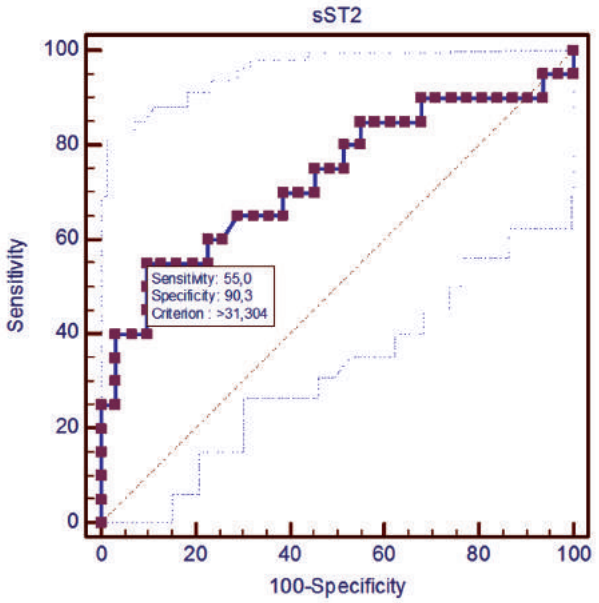

Fig. 1. Sensitivity and specificity of sST2 levels in the diagnosis of CMD (ROC-analysis)

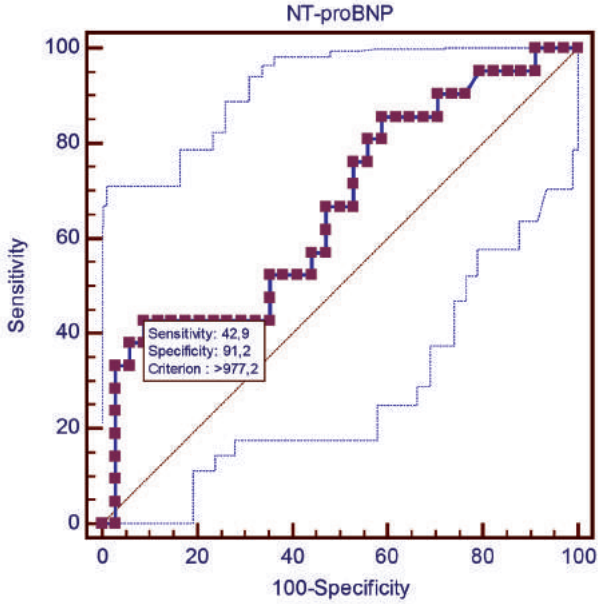

Fig. 2. Sensitivity and specificity of NT-proBNP levels in the diagnosis of CMD (ROC-analysis)

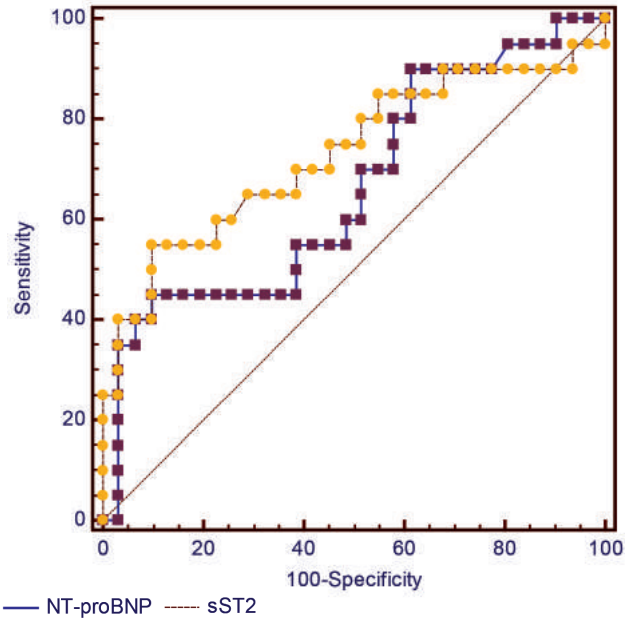

Fig. 3. Comparison of sensitivity and specificity of NT-proBNP and sST2 levels in the diagnosis of CMD (ROC-analysis)

DISCUSSION

This study demonstrated that patients with non-obstructive CAD and CMD had higher incidence of HFpEF than patients without CMD. The values of CFR were correlated with DD parameters and the concentrations of NT-proBNP and sST2. The levels of sST2 ≥ 31.304 ng / ml and NT-proBNP ≥ 977.2 pg / ml were identified as cut-off values for diagnosing CMD in patients with non-obstructive CAD.

HFpEF is one of the greatest problems in modern cardiology. Out of the estimated 5 million patients diagnosed with HF in the USA, approximately 50% have HFpEF [22]. In Europe, this proportion ranges from 22 to 73% [1]. Moreover, there is a growing understanding that HFpEF represents a heterogeneous syndrome with various phenotypes and comorbidities [23]. The results of a number of international studies using invasive or non-invasive diagnostic methods support the assumption that CMD occurs significantly more often than previously, including patients with HFpEF. V.L.Murthy et al. reported that 53% of patients with non-obstructive CAD and pain syndrome had evidence of inducible myocardial ischemia [11]. According to the latest meta-analysis data of 56 studies including 14,427 patients, the proportion of patients with CMD was 41% in the general population [12]. Moreover, when the prevalence of CMD was analyzed in patients with HFpEF, the incidence increased to 75–85% [13, 14]. Therefore, an innovative theory has been proposed recently according to which CMD represents “common soil” for the occurrence of both microvascular angina and HFpEF [4, 14, 24]. It is worth noting that patients with non-obstructive CAD,

despite preserved LVEF, are no less often subject to hospitalization due to HF decompensation [11].

CMD is a type of non-obstructive CAD in which small blood vessels feeding the cardiac muscle cannot cope with the load [14]. However, the potential mechanisms of CMD development have not yet been studied and include cellular metabolism disorders, systemic inflammation, reactive oxygen species (ROS) generation, increased coronary vasoconstrictor reactivity at the microvascular level, impaired endothelium-dependent and endothelium-independent vasodilator capacities, hormonal and electrolyte imbalance, etc., which results in development of fibrosis and increased myocardial stiffness and coronary microvascular resistance [12, 14–16, 25]. It is most likely that primary structural abnormalities in CMD are associated with damage to endothelial mitochondria and include their hyperplasia, reduction in size of organelles or their fragmentation, and structural damage, such as reduction of electron-dense matrix and disruption of inner and outer membranes [16, 23, 24]. The concomitant diseases, such as diabetes, hypertension, obesity, etc., not only activate superoxide overexpression via the mitochondrial electron-transport chain and NADPH oxidase, which partially contribute to impaired endothelial cell function, but also control parallel pathways causing the development of endothelial dysfunction [22]. It is worth noting, that endothelial dysfunction is one of the key mechanisms for the development and progression of CMD in HFpEF [18].

The endothelium plays a pivotal role in preventing platelet aggregation and leukocyte adhesion, regulating cell proliferation, and modulating vascular tone by synthesizing and releasing endothelium-derived relaxing factors, such as prostaglandins, nitric oxide (NO), and endothelium-dependent hyperpolarization (EDH) factors in different forms depending on the vessel size. NO predominantly mediates vasodilatation of relatively large coronary vessels, while EDH factors influence microvasculature resistance. As a consequence, alterations in both the myocytic and non-myocytic compartments can lead to the development of myocardial fibrosis and extracellular matrix remodeling and increase diastolic stiffness, which contributes to the progression of HFpEF [25, 26].

In the context of increased oxygen demand, impaired CFR, even in the absence of obstructive CAD, reflects myocardial ischemia at the microcirculatory level due to an imbalance in the ratio of oxygen demand and its delivery to the myocardium, which may predispose the myocardium to injury and impaired global ventricular mechanics and cardiac dysfunction [7]. Our data demonstrated that CMD was independently associated with DD parameters and the presence of HFpEF. This suggests that factors tipping the balance towards ischemic damage to cardiomyocytes in patients with existing CMD may impair LV function and increase the risk of HFpEF development, even in the absence of overt structural abnormalities or obstructive CAD. Thus, in the study including 385 patients with non-obstructive CAD, CMD was also significantly associated with echocardiography parameters of DD [27]. In patients with systolic dysfunction (LVEF < 35%) and non-obstructive CAD, CFR parameters were correlated with E / e' values [28]. In particular, microvascular endothelial dysfunction, decreased nitric oxide bioavailability, and increased profibrotic cytokine signaling may contribute to reduced coronary microvascular density or rarefaction and increased myocardial fibrosis, observed in HFpEF [7, 13, 14]. Correlation of CFR with biochemical markers of left ventricular volume overload, such as NT-proBNP (r = –0.290; p = 0.04), and cardiac fibrosis, such as sST2 (r = –0.331; p = 0.012), demonstrates a close relationship between these processes in the pathogenesis of HFpEF in patients without occlusive CAD.

In our study, only the levels of sST2 and NT-proBNP, but not MMP-9 and TIMP-1, were identified as cut-off values for diagnosing CMD in patients with non-obstructive CAD. Perhaps, in this population with non-obstructive CAD, the levels of sST2 reflect periarteriolar fibrosis that may occur in CMD [29]. In particular, CMD associated with chronic systemic inflammation may promote periarteriolar fibrosis and microvascular rarefaction, yielding decreased CFR, overexpression of sST2, and the development of HF symptoms and / or microvascular angina with "normal" CA [30]. In the study on mice models, decreased ST2 signaling with the progression of microvascular changes in the pressure overload state was associated with amplifying and sustaining arteriolar remodeling and periarteriolar fibrosis [29]. Furthermore, Aslan et al. (2019) established that serum sST2 levels were significantly higher in patients with microvascular angina compared with controls [31]. Hereinafter, chronic systemic inflammation causes adhesion and infiltration of monocytes and stimulation of integrated macrophages, which promotes myofibroblast differentiation and, eventually, collagen secretion leading to fibrosis and cardiac remodeling [10, 11, 32].

Thus, coronary microvascular ischemia may play an important role in the elevation of LV filling pressure, the development of DD, and the pathophysiology of HFpEF [8]. To support this fact, it was found that patients with CMD had higher levels of MMP-9 and TIMP-1 than those without it. But most likely, this process is secondary to CMD and is a consequence of HFpEF progression; therefore, these biomarkers did not show significance in the diagnosis of CMD, in contrast to sST2 and NT-proBNP.

CONCLUSION

It was established that CMD may play an important role in the pathogenesis of HFpEF. The values of CFR were correlated with DD parameters, and impaired CFR was associated with overexpression of cardiac biomarkers of fibrosis and remodeling. Serum levels of sST2 and NT-proBNP may be used as markers for non-invasive diagnosis of CMD.

REFERENCES

1. Ponikowski P., Voors A.A., Anker S.D., Bueno H., Cleland J.G., Coats A.J. 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. *Eur. Heart J.* 2016;18(8):891–975. DOI: 10.1002/ejhf.592.
2. Groenewegen A., Rutten F.H., Mosterd A., Hoes A.W. Epidemiology of heart failure. 2020;22(8):1342–1356. DOI: 10.1002/ejhf.1858.
3. McDonagh T.A., Metra M., Adamo M., Gardner R.S. et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) With the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur. Heart J.* 2021;42(36): 3599–3726. DOI: 10.1093/eurheartj/ehab368.
4. Tona F., Montisci R., Iop L., Civieri G. Role of coronary microvascular dysfunction in heart failure with preserved ejection fraction. *Rev. Cardiovasc. Med*. 2021;22(1):97–104. DOI: 10.31083/j.rcm.2021.01.277.
5. Vancheri F., Longo G., Vancheri S., Henein M. Coronary microvascular dysfunction. *J. Clin. Med.* 2020;9(9):2880. DOI: 10.3390/jcm9092880.
6. Crea F., Bairey Merz C.N., Beltrame J.F., Kaski J.C. et al. The parallel tales of microvascular angina and heart failure with preserved ejection fraction: a paradigm shift. *Eur. Heart J.* 2017;38(7):473–477. DOI: 10.1093/eurheartj/ehw461.
7. Taqueti V.R., Solomon S.D., Shah A.M., Desai A.S. et al. Coronary microvascular dysfunction and future risk of heart failure with preserved ejection fraction. *Eur. Heart J.* 2018;39(10):840–849. DOI: 10.1093/eurheartj/ehx721.
8. Rahman H., Scannell C.M., Demir O.M., Ryan M. et al. High-resolution cardiac magnetic resonance imaging techniques for the identification of coronary microvascular dysfunction. *JACC Cardiovasc. Imaging*. 2021 14(5):978–986. DOI: 10.1016/j.jcmg.2020.10.015.
9. Zavadovsky K.V., Mochula A.V., Boshchenko A.A. et al. Absolute myocardial blood flows derived by dynamic CZT scan vs invasive fractional flow reserve: Correlation and accuracy. *J. Nucl. Cardiol*. 2021;28(1):249–259. DOI: 10.1007/s12350-019-01678-z.;
10. Zavadovsky K.V., Mochula A.V., Maltseva A.N., Boshchenko A.A. et al. The diagnostic value of SPECT CZT quantitative myocardial blood flow in high-risk patients. *J. Nucl. Cardiol.* 2022; 29(3):1051–1063. DOI: 10.1007/s12350-020-02395-8.
11. Murthy V.L., Naya M., Foster C.R., Hainer J. et al. Improved cardiac risk assessment with noninvasive measures of coronary flow reserve. *Circulation.* 2011;124(20):2215–2224. DOI: 10.1161/CIRCULATIONAHA.111.050427.
12. Agostini D., Roule V., Nganoa C. et al. First validation of myocardial flow reserve assessed by dynamic 99mTc-sestamibi CZT-SPECT camera: head to head comparison with 15O-water PET and fractional flow reserve in patients with suspected coronary artery disease. The WATERDAY study. *Eur. J. Nucl. Med. Mol. Imaging*. 2018;45(7):1079–1090. DOI: 10.1007/s00259-018-3958-7.
13. Paulus W.J., Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J. Am. Coll. Cardiol*. 2013;62(4):263–271. DOI: 10.1016/j.jacc.2013.02.092.
14. Godo S., Suda A., Takahashi J., Yasuda S., Shimokawa H. Coronary microvascular dysfunction. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2021;41(5):1625–1637. DOI: 10.1161/ATVBAHA.121.316025.
15. Mileva N., Nagumo S., Mizukami T., Sonck J. et al. Prevalence of Coronary Microvascular Disease and Coronary Vasospasm in Patients With Nonobstructive Coronary Artery Disease: Systematic Review and Meta-Analysis. *J. Amer. Heart Ass*. 2022;11(7):e023207. DOI: 10.1161/JAHA.121.023207.
16. Zhou H., Toan S. Pathological roles of mitochondrial oxidative stress and mitochondrial dynamics in cardiac microvascular ischemia/reperfusion injury. *Biomolecules*. 2020;10(1):85. DOI: 10.3390/biom10010085.
17. Nagueh S.F., Smiseth O.A., Appleton C.P. 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. *Eur. Heart J. Cardiovasc. Imaging*. 2016;17(12):1321–1360. DOI: 10.1093/ehjci/jew082.
18. Henzlova M.J., Duvall W.L., Einstein A.J., Travin M.I., Verberne H.J. ASNC imaging guidelines for SPECT nuclear cardiology procedures: Stress, protocols, and tracers. *J. Nucl. Cardiol.* 2016;23 (3):606–639. DOI: 10.1007/s12350-015-0387-x.
19. Leppo J.A., Meerdink D.J. Comparison of the myocardial uptake of a technetium-labeled isonitrile analogue and thallium. *Circ. Res*. 1989;65(3):632–639. DOI: 10.1161/01.res.65.3.632.

20. Cerqueira M.D., Weissman N.J., Dilsizian V., Jacobs A.K. et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart: A statement for healthcare professionals from the cardiac imaging. *Circulation.* 2002;105(4):539–542. DOI: 10.1161/hc0402.102975.
21. Austen W.G., Edwards J.E., Frye R.L., Gensini G.G. et al. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. *Circulation.* 1975;51(4):5–40. DOI: 10.1161/01.cir.51.4.5.
22. Clark K.A.A., Velazquez E.J. Heart failure with preserved ejection fraction: time for a Reset. *JAMA.* 2020;324(15):1506–1508. DOI: 10.1001/jama.2020.15566.
23. Triposkiadis F., Butler J., Abboud F.M., Armstrong P.W., Adamopoulos S., Atherton J.J. et al. The continuous heart failure spectrum: moving beyond an ejection fraction classification. *Eur. Heart J.* 2019;40(26):2155–2163. DOI: 10.1093/eurheartj/ehz158.
24. Graziani F., Varone F., Crea F., Richeldi L. Treating heart failure with preserved ejection fraction: learning from pulmonary fibrosis. *Eur. J. Heart Fail*. 2018;20(10):1385–1391. DOI: 10.1002/ejhf.1286.
25. Zile M.R., Baicu C.F., Ikonomidis J.S., Stroud R.E., Nietert P.J. et al. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: contributions of collagen and titin. *Circulation*. 2015;131(14):1247–1259. DOI: 10.1161/CIRCULATIONAHA.114.013215.
26. Rahman H., Ryan M., Lumley M., Modi B. et al. Coronary microvascular dysfunction is associated with myocardial ischemia and abnormal coronary perfusion during exercise. *Circulation.* 2019;140:1805–1816. DOI: 10.1161/CIRCULATIONAHA.119.041595.
27. Kim I.C., Hwang J., Lee C.H., Cho Y.K. et al. Correlation of coronary microvascular function and diastolic dysfunction. *Eur. Heart J.* 2020;41(2):ehaa946.0903. DOI: 10.1093/ehjci/ehaa946.0903.
28. Snoer M., Monk-Hansen T., Olsen R.H., Pedersen L.R., Nielsen O.W. et al. Coronary flow reserve as a link between diastolic and systolic function and exercise capacity in heart failure. *Eur. Heart J. Cardiovasc. Imaging.* 2013;14(7):677–683. DOI: 10.1093/ehjci/jes269.
29. Garbern J.C., Williams J., Kristl A.C., Malick A., Rachmin I. et al. Dysregulation of IL-33/ST2 signaling and myocardial periarteriolar fibrosis. *J. Mol. Cell Cardiol*. 2019;128:179–186. DOI: 10.1016/j.yjmcc.2019.01.018.
30. Lewis G.A., Schelbert E.B., Williams S.G., Cunnington C., Ahmed F., McDonagh T.A. et al. Biological phenotypes of heart failure with preserved ejection fraction. *J. Am. Coll. Cardiol.* 2017;70(17):2186–2200. DOI: 10.1016/j.jacc.2017.09.006.
31. Aslan G., Polat V., Bozcali E., Opan S., Çetin N., Ural D. Evaluation of serum sST2 and sCD40L values in patients with microvascular angina. *Microvasc. Res*. 2019;122:85–93. DOI: 10.1016/j.mvr.2018.11.009.
32. Grakova E.V., Kopeva K.V., Teplyakov A.T., Ogurkova O.N., Soldatenko M.V., Garganeeva A.A. Clinical use of the new biomarker ST2 in patients with chronic heart failure of ischemic genesis: an exercise test. *Cardiovascular Therapy and Prevention.* 2019;18(4):12–18 (in Russ.). DOI: 10.15829/1728-8800-2019-4-12-18.

Authors information

Kopeva Kristina V. – Cand. Sci. (Med.), Researcher, Department of Myocardial Pathology, Cardiology Research Institute, Tomsk NRMC, Tomsk, http://orcid.org/0000-0002-2285-6438

Maltseva Alina N. – Post-Graduate Student, Laboratory for Radionuclide Research Methods, Cardiology Research Institute, Tomsk NRMC, Tomsk, http://orcid.org/0000-0002-1311-0378

Mochula Andrey V. – Cand. Sci. (Med.), Senior Researcher, Laboratory for Radionuclide Research Methods, Cardiology Research Institute, Tomsk NRMC, Tomsk, http://orcid.org/0000-0003-0883-466X

Grakova Elena V. – Dr. Sci. (Med.), Leading Researcher, Department of Myocardial Pathology, Cardiology Research Institute, Tomsk NRMC, Tomsk, http://orcid.org/0000-0003-4019-3735

Shipulin Vladimir V. – Cand. Sci. (Med.), Junior Researcher, Laboratory for Radionuclide Research Methods, Cardiology Research Institute, Tomsk NRMC, Tomsk, http://orcid.org/0000-0001-9887-8214

Gusakova Anna M. – Cand. Sci. (Biology), Researcher, Department of Clinical Laboratory Diagnostics, Cardiology Research Institute, Tomsk NRMC, Tomsk, http://orcid.org/0000-0003-0883-466X

Zavadovsky Konstantin V. – Dr. Sci. (Med.), Head of the Laboratory for Radionuclide Research Methods, Cardiology Research Institute, Tomsk NRMC, Tomsk, http://orcid.org/0000-0002-1513-8614.

(✉) **Kopeva Kristina V.,** Kristin-kop@inbox.ru

Received 17.03.2022;
approved after peer review 30.06.2022;
accepted 08.09.2022