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Potential role of peripheral blood mononuclear cell's mitochondrial respiratory dysfunction in heart failure severity prediction in patients with cardioverter-defibrillator implantation indications

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ABSTRACT

Background It has been reported that the mitochondrial respiratory dysfunction (MRD) is important mechanisms affecting the heart failure (HF) pathogenesis. We sought to evaluate the potential role of MRD of peripheral blood mononuclear cells (PBMC) in HF severity prediction in patients with cardioverter-defibrillator implantation indications.

Methods In this single-center study patients with HF of New York Heart Association (NYHA) I-III functional class (FC) and cardioverter-defibrillator implantation indications underwent transthoracic echocardiography (TTE) and MRD assessment using PBMC. Mitochondrial respiration rate (MRR) indicators (pyruvate + malate + adenosine diphosphate; succinate + adenosine diphosphate; pyruvate + malate – adenosine diphosphate [$V_{4.1}$]; succinate – adenosine diphosphate) were calculated. Correlations between HF NYHA FC, TTE and MRR indicators were evaluated. Based on our data, we developed a risk model regarding HF severity.

Results Of 53 (100.0%) HF patients, 33 (62.3%) had mild exercise intolerance (1st group) and 20 (37.7%) had moderate-to-severe exercise intolerance (2nd group). Patients with mild exercise intolerance were likely to have a higher $V_{4.1}$ ($P < 0.001$) values. $V_{4.1}$ was independently associated with moderate-to-severe exercise intolerance in univariate and multivariate logistic regression (OR = 0.932, 95% CI: 0.891–0.975, $P < 0.001$).

Conclusions The severity of HF is associated with PBMC mitochondrial respiratory dysfunction in patients with cardioverter-defibrillator implantation indications. Our HF severity risk model including $V_{4.1}$ parameters is able to distinguish patients with mild and moderate-to-severe exercise intolerance. Further investigations of their predictive significance are warranted.

The heart failure (HF) is a progressive clinical syndrome characterized by the inability of the heart to pump enough blood and oxygen to support the metabolic demands of other organs.[1] Chronic HF affects around 64 million of patients worldwide, and its prevalence is increasing due to the population ageing, the growing burden of comorbidities, and risk factors for HF.[2] In the European and the United States population, the prevalence of HF varies from 0.4% to 2.5%.[2,3] In the Russian Federation, according to recent years, it reaches 10.2%.[4]

Despite remarkable advances in the treatment of HF, mortality remains high and sudden cardiac death (SCD) accounts for roughly 40.0% of deaths in this population.[5] Prospective randomized studies of patients with HF have shown that implantable cardioverter-defibrillator (ICD) therapy translates into long-term clinical benefits, such as improved quality of life, increased functional capacity, reduction in hospitalization for HF, and SCD.[6,7]

Several studies have shown that the conventional echocardiographic indexes correlates with the outcome in HF patients.[8,9] The most common risk factors associated

with the severity of HF are the low 6 minute walk distance test (6MWDT), impaired left and right ventricular systolic and diastolic function, and the high level of right ventricle systolic pressure (RVSP) and HF biomarkers in the blood (brain natriuretic peptide, N-terminal natriuretic peptide type B, mid-regional proatrial natriuretic peptide, galectin 3, etc.).[10-12] However, there is still discussion about the most appropriate echocardiographic and laboratory indicator for predicting the severity of HF, and also about the cut-off value which should be used for the each parameter.

Another important risk factor for HF is the mitochondrial respiratory dysfunction (MRD). In recent years, the relationship between HF and mitochondrial function has received increasing attention. Researchers have noticed that MRD is widespread during HF.[13] Many findings have shown that cardiac myocardial mitochondria imbalances are involved in different cardiovascular diseases, including HF, and play a crucial role in their development.[14-16] Peripheral blood mononuclear cells (PBMC) are a group of mononuclear immune cells that exist in blood and exhibit important roles in the body's immune responses.[17] Among white blood cells, PBMC (lymphocytes and monocytes) depend on the oxidative metabolism of mitochondria, while neutrophils do not.[18] In addition, a recent report has demonstrated that the respiratory capacity of mitochondria in PBMC is reduced in asymptomatic patients with early HF, but the role of PBMC mitochondrial dysfunction in the development of HF remains fully unclear.[19]

The aim of this study was to evaluate the potential role of MRD of PBMC in HF severity prediction in patients with ICD implantation indications.

METHODS

Patient Population and Study Design

Patients with HF New York Heart Association (NYHA) functional class (FC) I-III and ICD implantation indications were included in this clinical, single-center, nonrandomized, cross-sectional observational study. The HF defined as complex of clinical syndrome with symptoms and signs that result from any structural (ischemic or nonischemic etiology) impairment of ventricular filling or ejection of blood. Individuals with NYHA FC of HF IV, recent myocardial infarction (MI) (less than 3 months), severe comorbidity (end-stage renal failure and hepatic insufficiency, cancer of any location), cognitive impairment, indications for the revascularization and heart transplantation, and patients under the age of 18 years were excluded.

All patients underwent the full physical (6MWDT, electrocardiography [ECG], transthoracic echocardiography [TTE], Holter ECG monitoring, coronary angiography, blood analyses) and additional (MRD assessment using PBMC) examination. All patients received the basic therapy in accordance with the present HF management guidelines.

Consent

The study was carried out in accordance with the principles of Helsinki Declaration and with the standards of Good Clinical Practice. The study protocol was approved by the Local Ethics Committee of the Cardiology Research Institute protocol No. 219 dated October 26, 2021. All participants received written informed consent prior to the study inclusion. Ethical approval by the hospital review committee and patient consent according to the institutional guidelines were obtained. All included patients were from a registered study (ClinicalTrials.gov, NCT03667989).

6-min Walk Distance Test

The assessment of HF FC was performed in accordance with the NYHA criteria, using 6MWDT. For the analysis, the walking distance in meters and NYHA FC were used, to which it corresponded: (1) More than 551 m: the patient had no signs of HF; (2): 426-550 m: I FC of HF; (3): 301-425 m – II FC of HF; (4): 151-300 m – III FC of HF; and (5) less than 150 m – IV FC of HF.

TTE Acquisition and Analysis

TTE with the intracardiac hemodynamic parameters assessment was performed using Philips HD15 PureWave (Netherlands) ultrasound machine. The examination was carried out from standard positions with the determination of the volume-dimensional and indexed indicators of the heart chambers, left ventricular ejection fraction (LVEF) and RVSP. The LVEF was evaluated using Simpson method (biplane method of disks). The left ventricular end-systolic (LVESI) and end-diastolic (LVEDI), right (RAI) and left atrial (LAI) indexes were calculated using the ratio of chamber (left ventricle, right atrium or left atrium) volume to the body surface area. The

left ventricular sphericity index (LVSI) was calculated using the ratio of the end-diastolic dimension to the longitudinal dimension of the left ventricle in diastole. The index of myocardial mass (IMM) was calculated using formula: 0.8 x (1.05 x [left ventricular end-diastolic dimension + interventricular septum thickness + left ventricle posterior wall thickness]^3 – left ventricular end-diastolic dimension^3) + 0.6. The mitral, tricuspid and aortic valves functions, as well as the right and left ventricles contractility, were assessed.

Mitochondrial Respiratory Function Data Acquisition

Blood sampling was carried out no more than 1 h before ICD implantation. Patients were sampled venous blood (9 mL) in ethylenediaminetetraacetic acid (EDTA) vacutainers. The PBMC fraction was isolated on a Histopaque-1077 density gradient (Sigma). Resulting ring containing mononuclear cells was washed in phosphate-buffered saline (Sigma). The cell pellet was undergone to osmotic lysis by introduction into a sucrose medium (0.25 M sucrose with EDTA) and subsequent soft pipetting to release the mitochondria into the solution. Mitochondria were further isolated by differential centrifugation in sucrose medium.[20] The resulting pellet was resuspended in a minimum volume of 0.25 M sucrose for further analysis.

The activity of respiratory processes of isolated mitochondria was determined in follow pre-oxygenated incubation media (in mM): sucrose – 250.0; KCl – 10.0; KH_2PO_4 – 5.0; $MgCl_2$ – 1.25; HEPES – 5.0; pyruvate – 6.0 + malate – 8.0 or succinate – 5.0; pH = 7.35-7.40. The studies were carried out in a thermostated cell (1 mL) under constant stirring with a magnetic stirrer and a temperature of 25-27 °C. The oxygen concentration in the incubation medium was measured amperometrically using a Clarke-type electrode connected to Expert – 001 liquid analyzer (Econix-Expert, Russia). The oxygen consumption rate was calculated in nM O_2/min/mg mitochondrial protein (MP). The conjugation of the processes of oxidation and phosphorylation was assessed based on the respiratory control coefficient, the calculation formula of is as follows V_3/V_4, where V_3 is the oxygen consumption rate by mitochondria in the medium in the presence of the oxidation substrate, pyruvate-malate mixture or succinate, and the phosphorylation substrate, adenosine diphosphate (ADP) (200 μM); V_4 is the oxygen consumption rate after the exhaustion of ADP in the incubation medium.[20] Mitochondrial respiration rate (MRR) indicators (pyruvate + malate + ADP - $V_{3.1}$; succinate + ADP - $V_{3.2}$; pyruvate + malate–ADP-$V_{4.1}$; succinate–ADP-$V_{4.2}$) were calculated (Figure 1).

Clinical Evaluation and Study Design

Information on clinical assessment was obtained for 53 patients who were included in a cross-sectional observational study. After the assessment of HF FC in accordance with 6MWDT results, all patients were divided into two groups. The 1st group included patients with mild exercise intolerance. The 2nd group included patients with moderate-to-severe exercise tolerance. The flow chart of the study is shown in Figure 2.

Statistical Analysis

All statistical analyses were performed with software package Statistica 10.0, StatSoft (USA) and Medcalc 19.2.6 statistical software package (USA), and statistical significance was defined by a *P*-value < 0.05. Categorical variables are presented as numbers (*n*) and percentages (%), and continuous variables either as mean ± SD for normally distributed variables or as median (M_e) and interquartile ranges [Q_1; Q_3] for non-normally distributed variables. The distribution of continuous data was tested for normality using Kolmogorov-Smirnov, Lilliefors and Shapiro-Wilk tests. Group differences of continuous data were analyzed using the two-sided Student's *t*-test for normally distributed data or the Mann–Whitney *U*-test for independent ordinal or non-normally distributed data. The distribution of categorical variables was analyzed using chi-square or Fisher's exact test. A comparison between patients with mild and moderate-to-severe exercise intolerance was performed for the complete dataset.

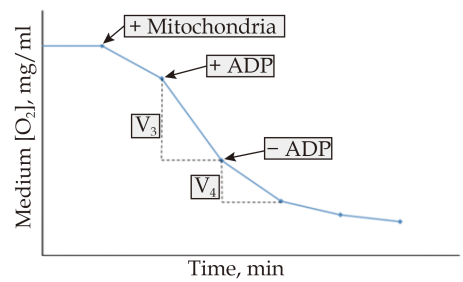

Figure 1 Mitochondrial respiration rate acquisition in oxidation substrate (pyruvate-malate mixture or succinate). State 3 or active phosphorylating state (V_3) is observed in the presence of oxygen, oxidation substrate (pyruvate-malate, succinate) and ADP. State 4 or the non-phosphorylating state (V_4) is characterized by inhibition of respiration due to the depletion of ADP. ADP: adenosine diphosphate.

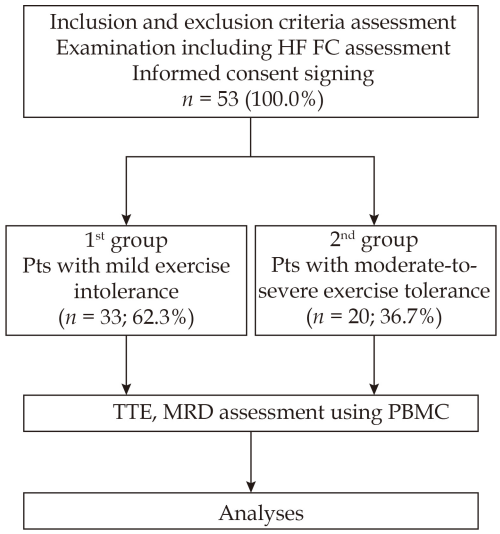

Figure 2 Study flow chart. HF: heart failure; FC: functional class; TTE: transthoracic echocardiography; NYHA: New York Heart Association; MRD: mitochondrial respiratory dysfunction; PBMC: peripheral blood mononuclear cells; Pts: patients.

Risk Stratification and Predictive Model Development

Binary logistic regression analysis was used to identify variables associated with the moderate-to-severe exercise intolerance. Before multivariate analysis, multicollinearity was excluded using Spearman analyses. After checking for significant results in univariate analyses, a multivariate logistic regression was performed. Stepwise elimination technique was used to find independently associated parameters with the moderate-to-severe exercise intolerance. Only variables with $P < 0.05$ were included in the final model. Goodness-of-fit was assessed by the Hosmer-Lemeshow test. Significant correlations between the predictors and other measurements were examined using Pearson correlation and *t*-test. Results from our regression analysis were presented as odds ratios (OR) with 95% confidence intervals (CI). Odds ratios and the beta coefficients used in the logistic equation could be converted into each other: $OR = e^{\beta}$.

In the end, one parameter ($V_{4.1}$), independently associated with the moderate-to-severe exercise intolerance criteria, were included in the predictive model. The area under the curve (AUC) was calculated to evaluate the discriminatory ability of the risk stratification score.

RESULTS

Patient's Demographic and Clinical Data

Total 53 (100.0%) patients were included in the study. For all patients, the mean age was 59.4 ± 11.3 years and 37 (69.8%) patients were males. The 1st group included 33 (62.3%) patients with mild exercise intolerance. The 2nd group included 20 (36.7%) patients with moderate-to-severe exercise intolerance. The demographics and clinical characteristics of patients are shown in Table 1. Patients with moderate-to-severe exercise intolerance were more likely to have diagnosed pulmonary hypertension (45.0% *vs.* 6.1%, $P = 0.018$). Also, in patients with mild exercise intolerance value of 6MWDT was significantly higher (415.1 ± 76.3 *vs.* 242.2 ± 60.7 m, $P < 0.001$). This significant difference was due to the fact that patients were divided into groups according to the results of 6MWDT.

Transthoracic Echocardiography Characteristics

TTE revealed a significantly higher right atrial volume (RAV) [95.6 (74.6–110.2) *vs.* 67.3 (51.5–90.7) mL, $P = 0.022$] and index (RAI) [49.0 (37.8–55.3) *vs.* 35.1(26.7–44.8) mL/m^2, $P = 0.006$], right ventricle (RV) size (26.9 ± 4.2 *vs.* 24.2 ± 2.7 mm, $P = 0.022$) and RVSP [34.5 (30.5–53.5) *vs.* 29.0 (25.0–34.0) mmHg, $P = 0.003$] in patients with moderate-to-severe exercise intolerance. According to TTE results in patients with mild exercise intolerance values of stroke volume (SV) (74.9 ± 18.9 *vs.* 63.7 ± 15.4 mL, $P = 0.033$) and LVEF [35.0 (30.0–47.0) % *vs.* 28.0 (25.0–33.5) %, $P = 0.001$] were significantly higher. A detailed comparison of TTE parameters between patients with mild and moderate-to-severe exercise intolerance are presented in Table 2.

Mitochondrial Respiratory Dysfunction Characteristics

Assessment of MRD revealed a significantly higher $V_{3.1}$ [137.5 (105.7–187.5) nM O_2/min/mg MP *vs.* 85.8 (59.2–118.6) nM O_2/min/mg MP, $P = 0.002$] and $V_{4.1}$ [52.3 (45.3–86.9) n O_2/min/mg MP *vs.* 26.1 (19.3–37.8) nM O_2/min/mg MP, $P < 0.001$] values in patients with mild exercise intolerance. A detailed comparison of MRR parameters between groups is presented in Table 3.

Analysis for HF Severity Risk Stratification

Based on the evaluated parameters, $V_{4.1}$ was the parameter most strongly associated with the moderate-to-severe exercise intolerance. Its discriminative ability was assessed by ROC analysis revealing an AUC of 0.864 (95% CI: 0.741–0.942). In addition, this parameter strongly correlated with the $V_{3.1}$ ($R = 0.871$, $P < 0.001$). The ability of LVEF to discriminate for the moderate-to-severe exercise intolerance was similar with an AUC of 0.757 (95% CI:

0.619–0.864). Concerning RVSP, RV size, SV, RAI and $V_{3.1}/V_{4.1}$ ratio, calculation of the ROC curves revealed an AUC of 0.742 (95% CI: 0.604–0.853), 0.689 (95% CI: 0.547–0.809), 0.677 (95% CI: 0.534–0.799), 0.724 (95% CI: 0.584–0.838) and 0.677 (95% CI: 0.535–0.799), respectively. In addition, RVSP strongly correlated with the pulmonary hypertension (R = 0.702, $P < 0.001$) and RAI with RAV (R = 0.963, $P < 0.001$).

According to the univariate logistic regression analysis $V_{4.1}$ (OR = 0.932, 95% CI: 0.891–0.975, $P < 0.001$), LVEF (OR = 0.911, 95% CI: 0.847–0.980, $P = 0.002$), RVSP (OR = 1.093, 95% CI: 1.026–1.164, $P = 0.001$), RV size (OR = 1.282, 95% CI: 1.045–1.571, $P = 0.006$), SV (OR = 0.963, 95% CI: 0.930–0.997, $P = 0.026$), RAI (OR = 1.056, 95% CI: 1.010–1.103, $P = 0.006$) and $V_{3.1}/V_{4.1}$ ratio (OR = 2.390, 95% CI: 1.133–5.041, $P = 0.010$) were independently associated with the moderate-to-severe exercise intolerance. Only $V_{4.1}$ parameters remained significant in multivariate regression, even after adjustment for age, female gender, body mass index (BMI), estimated glomerular filtration rate (eGFR), presence of MI in anamnesis and QRS duration (OR = 0.932, 95% CI: 0.891–0.975). The beta coefficients in the logistic equation correspond to the natural logarithm of the Odds ratio ($\ln(OR) = \beta$; $OR = e^{\beta}$).

Severity of HF Risk Score Development

A risk score for the severity of HF was created using logistic regression based on collected data. The $V_{4.1}$ parameter was included in the final model since this parameter remained significant in multivariate logistic regression, even after adjustment for age, female gender, BMI, eGFR, presence of MI in anamnesis and QRS duration. The AUC was calculated to evaluate the discriminatory ability of the risk stratification score. The model demonstrated discrimination ability evidenced by an AUC of 0.864 (Figure 3). At a cutoff value of > 0.35; the model exhibited a sensitivity of 85.00% and a specificity of 84.85% in distinguishing of patients with ICD implantation indications regarding the HF severity. Our risk stratification analysis revealed a positive predictive value of 70.00% and a negative predictive value of 87.88%.

The $V_{4.1}$ value must be inserted in the score equation (in nmol O_2/min/mg MP). The result of the logistic equation below is the probability (p) of the moderate-to-severe exercise intolerance. If the score is > 0.35, this risk score permits the identification of patients with ICD implantation indications at an increased risk for the moderate-to-

Table 1 Demographic and clinical characteristics of enrolled patients.

Demographic and clinical characteristics	Total (n = 53)	1st group Mild exercise intolerance (n = 33)	2nd group Moderate-to-severe exercise intolerance (n = 20)	$P^{2\text{-}3}$
	1	2	3	
Age, yrs	59.4 ± 11.3	58.5 ± 10.2	61.1 ± 13.1	0.468
Male gender	37 (69.8%)	22 (66.6%)	15 (75.0%)	0.620
History of MI	20 (37.7%)	11 (33.3%)	9 (45.0%)	0.485
History of CA stenting	16 (30.2%)	9 (27.3%)	7 (35.0%)	0.646
History of CABG	4 (7.5%)	2 (6.1%)	2 (10.0%)	0.818
Ischemic HF	22 (41.5%)	12 (36.4%)	10 (50.0%)	0.485
Nonischemic HF	31 (58.5%)	21 (63.6%)	10 (50.0%)	0.451
Pulmonary hypertension	11 (20.7%)	2 (6.1%)	9 (45.0%)	**0.018**
6MWDT, m	349.9 ± 109.9	415.1 ± 76.3	242.2 ± 60.7	**< 0.001**
Arrhythmias and heart conduction system diseases prior to ICD implantation				
SVT	14 (26.4%)	5 (15.1%)	9 (45.0%)	0.072
Ventricular fibrillation	2 (3.8%)	0	2 (10.0%)	0.550
Atrial fibrillation	15 (28.3%)	9 (27.3%)	6 (30.0%)	0.876
LBBB	34 (64.1%)	21 (63.6%)	13 (65.0%)	0.941
RBBB	4 (7.5%)	1 (3.0%)	3 (15.0%)	0.474
QRS duration, ms	149.2 ± 29.4	145.1 ± 30.5	156.1 ± 26.9	0.079
$_{C}$QT, ms	470.7 ± 40.9	467.3 ± 38.1	476.2 ± 45.7	0.485

Continued

Demographic and clinical characteristics	Total (n = 53)	1st group Mild exercise intolerance (n = 33)	2nd group Moderate-to-severe exercise intolerance (n = 20)	$P^{2\text{-}3}$
	1	2	3	
ICD implantation indications				
SCD primary prevention	39 (73.6%)	28 (84.9%)	11 (55.0%)	0.072
SCD secondary prevention	14 (26.4%)	5 (15.1%)	9 (45.0%)	0.072
Comorbidities				
Hypertension	21 (39.6%)	13 (39.4%)	8 (40.0%)	0.769
Diabetes mellitus	16 (30.2%)	10 (30.3%)	6 (30.0%)	0.992
Obesity	21 (39.6%)	14 (42.4%)	7 (35.0%)	0.659
Stroke	2 (3.8%)	2 (6.1%)	0	0.720
Dyslipidemia	20 (37.7%)	14 (42.4%)	6 (30.0%)	0.457
eGFR, mL/min per 1.73 m^2	72.0 (63.0–89.0)	78.0 (63.0–86.0)	71.0 (64.5–92.0)	0.679
Body mass index	28.6 ± 5.4	28.9 ± 4.7	28.3 ± 6.5	0.508
Therapy				
Beta-blockers	50 (94.3%)	32 (96.9%)	18 (90.0%)	0.679
ACEI	16 (30.2%)	11 (33.3%)	5 (25.0%)	0.620
Amiodarone	21 (39.6%)	13 (39.4%)	8 (40.0%)	0.978
AIIR blocker	36 (67.9%)	21 (63.6%)	15 (75.0%)	0.497
Statins	41 (77.3%)	25 (75.7%)	16 (80.0%)	0.804
Potassium-sparing diuretics	44 (83.0%)	27 (81.8%)	17 (85.0%)	0.854
Loop diuretics	24 (45.3%)	14 (42.4%)	10 (50.0%)	0.653
Anticoagulants	21 (39.6%)	13 (39.4%)	8 (40.0%)	0.978
Antiplatelet agents	28 (52.8%)	17 (51.5%)	11 (55.0%)	0.840
¡SGLT2	39 (73.6%)	21 (63.6%)	18 (90.0%)	0.112

Bold values indicate statistical significance. Values are expressed as Mean ± SD or M_e (Q_1; Q_3) for continuous variables and n (%) for categorical variables. 6MWDT: 6 minute walk distance test; ACEI: angiotensin-converting enzyme inhibitors; AIIR: angiotensin II receptor; BMI: body mass index; CA: coronary artery; CABG: coronary artery bypass grafting; $_C$QT: corrected QT interval; eGFR: estimated glomerular filtration rate; ICD: implantable cardioverter-defibrillator; ¡SGLT2: inhibitors of sodium-glucose co-transporter 2; HF: heart failure; LBBB: left bundle branch block; LV: left ventricle; MI: myocardial infarction; RBBB: right bundle branch block; SCD: sudden cardiac death; SVT: sustained ventricular tachycardia.

Table 2 Transthoracic echocardiography parameters of enrolled patients.

TTE parameters	Total (n = 53)	1st group Mild exercise intolerance (n = 33)	2nd group Moderate-to-severe exercise intolerance (n = 20)	$P^{2\text{-}3}$
	1	2	3	
LA size, mm	45.9 ± 5.2	45.3 ± 5.2	46.8 ± 5.2	0.430
RV size, mm	25.2 ± 3.6	24.2 ± 2.7	26.9 ± 4.2	0.022
IVS, mm	10.1 ± 1.8	10.1 ± 1.7	10.1 ± 2.1	0.963
LVPW, mm	10.3 ± 1.7	10.3 ± 1.8	10.4 ± 1.6	0.383
LVEDD, mm	63.1 ± 8.9	62.7 ± 9.9	63.8 ± 7.0	0.686
LVESD, mm	52.3 ± 10.9	51.2 ± 12.1	54.2 ± 8.5	0.424
LVEDV, mL	215.0 (148.0–254.0)	188.0 (137.0–262.0)	224.0 (183.5–253.5)	0.255
LVESV, mL	145.0 (96.0–182.0)	113.0 (82.0–182.0)	166.0 (136.5–186.0)	0.078
SV, mL	70.7 ± 18.3	74.9 ± 18.9	63.7 ± 15.4	0.033

Continued

TTE parameters	Total (n = 53)	1st group Mild exercise intolerance (n = 33)	2nd group Moderate-to-severe exercise intolerance (n = 20)	P^{2-3}
	1	2	3	
LVEF, %	33.0 (27.0–43.0)	35.0 (30.0–47.0)	28.0 (25.0–33.5)	0.001
CI, L/min/m^2	2.40 ± 0.69	2.40 ± 0.64	2.23 ± 0.76	0.075
LAV, mL	101.9 (84.6–127.9)	96.4 (82.6–119.1)	114.6 (92.7–130.2)	0.265
RAV, mL	80.7 (58.4–100.3)	67.3 (51.5–90.7)	95.6 (74.6–110.2)	0.022
LVSI	0.63 (0.56–0.68)	0.63 (0.55–0.68)	0.63 (0.57–0.68)	1.000
RVSP, mmHg	31.0 (27.0–37.0)	29.0 (25.0–34.0)	34.5 (30.5–53.5)	0.003
E, cm/s	65.0 (50.0–80.0)	64.0 (50.0–75.0)	66.0 (48.0–93.5)	0.479
A, cm/s	67.0 (53.0–73.0)	67.0 (55.0–73.0)	67.5 (50.0–73.5)	0.963
E/A	1.00 (0.72–1.38)	0.97 (0.72–1.17)	1.12 (0.71–1.83)	0.491
RVS, cm/m^2	1.80 ± 0.19	1.78 ± 0.17	1.83 ± 0.23	0.550
RAA, cm/m^2	1.80 ± 0.23	1.77 ± 0.19	1.83 ± 0.28	0.526
LAI, mL/m^2	52.0 (44.3–62.7)	50.5 (40.6–60.9)	59.1 (47.6–67.7)	0.098
RAI, mL/m^2	40.8 (30.6–49.8)	35.1 (26.7–44.8)	49.0 (37.8–55.3)	0.006
LVEDI, mL/m^2	105.5 (75.4–127.9)	102.2 (74.5–125.6)	120.0 (91.2–138.2)	0.174
LVESI, mL/m^2	69.9 (47.3–95.2)	59.2 (41.6–92.6)	86.1 (64.8–101.2)	0.057
IMM, g/m^2	128.8 ± 37.3	122.2 ± 28.8	139.7 ± 46.9	0.291

Bold values indicate statistical significance. Values are expressed as Mean ± SD or M_e (Q_1; Q_3). A: left ventricle active filling rate; CI: cardiac index (B); E: left ventricle early diastolic filling rate; E/A: left ventricle early diastolic filling rate to left ventricle active filling rate ratio; IMM: index of myocardial mass; IVS: interventricular septum; LA: left atrium; LAI: left atrial index; LAV: left atrial volume; LVEDD: left ventricular end-diastolic dimension; LVEDI: left ventricular end-diastolic index; LVEDV: left ventricular end-diastolic volume; LVEF: left ventricle ejection fraction; LVESD: left ventricular end-systolic dimension; LVESI: left ventricular end-systolic index; LVESV: left ventricular end-systolic volume; LVPW: left ventricle posterior wall; LVSI: left ventricular sphericity index; RAA: ratio of ascending aortic diameter to body surface area; RAI: right atrial index; RAV: right atrial volume; RV: right ventricle; RVS: ratio of the Valsalva sinus diameter to the body surface area; RVSP: right ventricle systolic pressure; SV: stroke volume.

Table 3 Mitochondrial respiration rate parameters of enrolled patients.

MRR parameters	Total (n = 53)	1st group Mild exercise intolerance (n = 33)	2nd group Moderate-to-severe exercise intolerance (n = 20)	P^{2-3}
	1	2	3	
$V_{3.1}$, nmol O_2/min/mg	121.8 (76.4–170.1)	137.5 (105.7–187.5)	85.8 (59.2–118.6)	0.002
$V_{3.2}$, nmol O_2/min/mg	119.0 (70.5–170.6)	128.7 (74.2–190.4)	114.0 (62.3–156.4)	0.335
$V_{4.1}$, nmol O_2/min/mg	45.9 (30.4–66.6)	52.3 (45.3–86.9)	26.1 (19.3–37.8)	< 0.001
$V_{4.2}$, nmol O_2/min/mg	41.9 (25.8–69.8)	44.6 (31.2–73.5)	35.3 (22.3–51.2)	0.086
RCC_1 ($V_{3.1}$/ $V_{4.1}$)	2.51 (2.08–3.26)	2.37 (2.06–2.68)	2.94 (2.43–3.47)	0.032
RCC_2 ($V_{3.2}$/ $V_{4.2}$)	2.63 (2.15–3.00)	2.63 (2.14–2.90)	2.67 (2.20–3.41)	0.446

Bold values indicate statistical significance. Values are expressed as M_e (Q_1; Q_3). MP, mitochondrial protein; MRR, mitochondrial respiration rate; RCC_1, respiratory control coefficient $V_{3.1}$/ $V_{4.1}$ ratio; RCC_2, respiratory control coefficient $V_{3.2}$/ $V_{4.2}$ ratio; $V_{3.1}$, oxygen consumption rate by mitochondria in pyruvate, malate and adenosine diphosphate; $V_{3.2}$, oxygen consumption rate by mitochondria in succinate and adenosine diphosphate; $V_{4.1}$, oxygen consumption rate by mitochondria in pyruvate and malate; $V_{4.2}$, oxygen consumption rate by mitochondria in succinate.

severe exercise intolerance and following intensified therapy.

Equation (1): Probability (P) of the moderate-to-severe exercise intolerance.

$$P = \frac{1}{1+e^{-z}} \quad z = 2.57 - 0.06 \times V4.1\,[\text{nmol O}_2/\text{min/mg MP}]$$

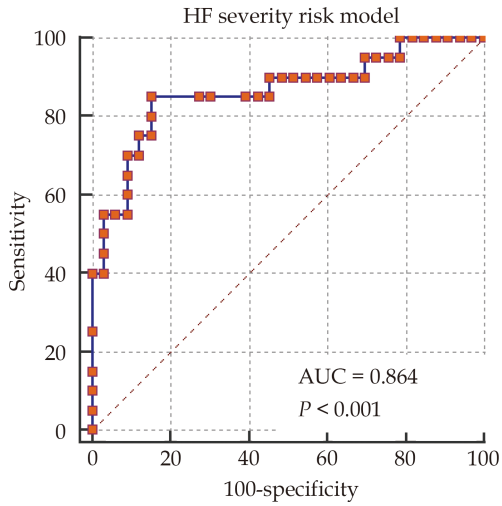

Figure 3 Risk model of moderate-to-severe exercise intolerance. AUC: area under the curve; HF: heart failure.

DISCUSSION

To our knowledge, the present results provide the first evidence of an association between the severity of HF with peripheral cellular MRD in patients with ICD implantation indications. The major findings of this study are that in patients with moderate-to-severe exercise intolerance mitochondrial respiration rate is markedly reduced compared to that in patients with mild exercise intolerance.

Old age, high BMI, low eGFR, wide QRS duration, MI history and low LVEF are well-known major risk factors for HF.[21-24] In our study, patients with moderate-to-severe exercise intolerance (2nd group) compared with mild exercise intolerance (1st group) did not differ significantly in the above HF risk factors, with the exception of LVEF. In the 1st group patients LVEF was significantly higher ($P = 0.001$). According to the univariate logistic regression analysis LVEF was independently associated with the moderate-to-severe exercise intolerance (OR = 0.911, 95% CI: 0.847–0.980, $P = 0.002$). But, the multivariate logistic regression analysis showed that none of these factors was associated with the moderate-to-severe exercise intolerance.

The RVSP is a readily available TTE parameter which goes up with the age and weight gain.[10] Echocardiographic findings from the 1097 participants enrolled in PARAGON-HF demonstrate that higher RVSP is associated with heightened risk for HF hospitalization.[24] According to our study results, RVSP in patients with NYHA FC III was significantly higher than in patients with NYHA FC I-II ($P = 0.003$). Furthermore, univariate logistic regression showed that RVSP is one of the independent predictors of the moderate-to-severe exercise intolerance (OR = 1.093, 95% CI: 1.026–1.164, $P = 0.001$). In addition, patients between groups differed in other TTE parameters (RV size, SV and RAI) and the univariate logistic regression analysis showed that RV size (OR = 1.282, 95% CI: 1.045–1.571, $P = 0.006$), SV (OR = 0.963, 95% CI: 0.930–0.997, $P = 0.026$) and RAI (OR = 1.056, 95% CI: 1.010–1.103, $P = 0.006$) were independently associated with the moderate-to-severe exercise intolerance. But, the multivariate logistic regression analysis showed that none of these factors was associated with the moderate-to-severe exercise intolerance.

It has been shown that the MRD is involved in HF.[25,26] The mitochondrial respiration is essential for PBMC homeostasis and its dysfunction can lead to the numerous inflammatory responses in various diseases.[27] Recent studies in HF have focused on the inflammation and systemic oxidative stress potentially related to the MRD of PBMC.[19,28] These cells are involved in many inflammatory diseases, including those caused by ischemia-reperfusion episodes, which play a key role in cardiovascular changes.[29] Whether their MRD can be used as a diagnostic, severity and/or prognosis biomarker in cardiovascular diseases is under evaluation.

The first data were reported by Li, *et al.* who described a global decrease in mitochondrial respiratory function in 25 early stages, asymptomatic HF patients, as compared to 24 controls.[19] The high sensitivity C-reactive protein, interleukin-6 and tumor necrosis factor-α, were significantly higher, and superoxide dismutase - a major antioxidant factor-transforming superoxide anion into hydrogen peroxide, was reduced in HF patients. Then, Shirakawa *et al.* observed in 31 compensated HF patients with reduced LVEF < 35% that the activities of several MRR complexes were altered.[28] Thus, complexes I + II and the maximal electron transfer system with complexes I + II and II alone were significantly reduced in FC NYHA III (16 patients) versus the NYHA I or II groups (15 patients), $P < 0.05$. This suggests that impaired PBMC mitochondrial respiration is related to the degree of severity of the disease. At the same time, mitochondrial reactive oxygen species were significantly higher in the NYHA III group than in the NYHA I or II groups. Recently, data from 19 severe HF stage patients also showed an impairment of maximal respiration as compared to the controls, with the basal oxygen consumption rate tending to be lower.[30] According to our study results, in pati-

ents with moderate-to-severe exercise intolerance, the MRD indicators such as $V_{3.1}$ ($P = 0.002$) and $V_{4.1}$ ($P < 0.001$) and were significantly lower compared with mild exercise intolerance. But $V_{3.2}$ was not decreased significantly in the 2nd group compared to the 1st group ($P = 0.335$). The results obtained may reflect metabolic changes associated with a decrease in respiration during the pyruvate and malate oxidation (respiratory chain complex I activity) and the absence of such during succinate oxidation. The latter may be associated with cell compensatory reactions of against the background of prolonged systemic exposure to hypoxia on circulating cells in HF, greater stability of succinate dehydrogenase (respiratory chain complex II), as well as a shorter path of electron transfer in the respiratory chain, which is less reflected in the oxygen consumption rate decrease and ATP synthesis, respectively. The RCC_1 ($V_{3.1}/V_{4.1}$) was rather increased in the 2nd group, which was opposite to the result of the $V_{3.1}$. The revealed differences may be due to HF duration. Thus, in the 2nd group, protective mechanisms activated in PBMC aimed at reducing oxygen consumption rate in the V_4 metabolic state to reduce electron leakage from the respiratory chain and potentially reduce the synthesis of reactive oxygen species. The univariate and multivariate logistic regression showed that the $V_{4.1}$, even after adjustment for age, female gender, BMI, eGFR, presence of MI in anamnesis and QRS duration, was the only independent predictor of moderate-to-severe exercise intolerance (OR = 0.932, 95% CI: 0.891–0.975). This may indicate that the assessment of MRD may also be an interesting marker of HF severity and be considered as useful signaling factors. But differences of opinion with previous studies highlight the importance of these findings and the need for future large-scale studies.

Thus, to better understand whether circulating PBMC mitochondrial function in patients with HF may be indicative of diagnostics, prognosis, multicenter clinical trials are needed in which a large number of HF patients are studied using a standardized analysis of PBMC mitochondrial function measured along with various blood biomarkers and clinical outcomes, and using linear regression modeling to control for individual comorbid conditions or therapeutic utility.[19,27,28] In our study, $V_{4.1}$ parameter reflecting HF severity processes was selected for our risk stratification model. This parameter appears to be able to predict moderate-to-severe exercise intolerance in patients with ICD implantation indications. The developed HF severity risk stratification model allowed us to correctly predict the moderate-to-severe exercise intolerance in our study cohort with an accuracy of 81.13%. The sensitivity of the model was 85.00%, while the specificity was 84.85%. The positive predictive value was 70.00%, while the negative predictive value was 87.88%. Thus, a negative result from our risk stratification model (below the cut-off value) can be used to rule out the mild HF. The negative predictive value is of particular importance in clinical applications. Patients scoring above the cut-off value have an increased risk for the moderate-to-severe exercise intolerance and they should intensify therapy.

Limitations

Our study has some limitations. One of these is non-randomized and single-center type of study. Limitations of the study also included the absence of follow-up and relatively small sample size, which may have reduced thevalue of the results.

Conclusions

In summary, HF severity is associated with PBMC mitochondrial respiratory dysfunction in patients with ICD implantation indications. We have developed HF severity risk model including $V_{4.1}$ parameters is able to distinguish patients with mild and moderate-to-severe exercise intolerance. Further investigations of their predictive significance are warranted.

DECLARATIONS

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Disclosures

The Authors declares that there is no conflict of interest.

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