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Functional iron deficiency and diastolic function in heart failure with preserved ejection fraction

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ABSTRACT

Background: Functional iron deficiency (FID) is an independent risk factor for poor outcome in advanced heart failure with reduced EF, but its role in heart failure with preserved EF (HFPEF) remains unclear. We aimed to investigate the impact of FID on cardiac performance determined by pressure–volume loop analysis in HFPEF.
Methods: 26 HFPEF patients who showed an increase in LV stiffness by pressure–volume (PV) loop analysis obtained by conductance-catheterization, performed exercise testing, echocardiographic examination including tissue Doppler and determination of iron metabolism: serum iron, ferritin and transferrin saturation. HFPEF patients who provided ferritin <100 μg/l or ferritin of 100–299 μg/l in combination with transferrin saturation <20% were defined as having FID. In 14 patients the expression of transferrin receptor was determined from available endomyocardial biopsies.
Results: Fifteen out of 26 HFPEF patients showed FID without anemia. Compared to control subjects and HFPEF patients without FID, HFPEF patients with FID showed an up-regulation of the myocardial transferrin receptor expression ($p < 0.05$). No differences between HFPEF patients with and without iron deficiency were found in heart dimensions, systolic and diastolic function obtained by PV-loop and echocardiography analysis. According to the linear regression analysis, LV stiffness was correlated with peak oxygen uptake ($r = -0.636$, $p < 0.001$) but not with the ferritin level or transferrin saturation. No relation was found between FID and exercise capacity. The association of LV stiffness with exercise performance was independent from the level of iron deficiency.
Conclusion: In non-anemic HFPEF patients, cardiac dysfunction and impaired exercise capacity occur independently of FID.

1. Introduction

Heart failure despite preserved ejection fraction (HFPEF) accounts for about 50% of all heart failure patients [1] and their prognosis compares to that of patients with systolic heart failure [2]. Risk factors for the development of HFPEF are multifactorial and also depend on quantity and quality of comorbidities including arterial hypertension, diabetes mellitus, kidney and lung disease, and anemia. In addition, a high rate of mortality in HFPEF does not include direct cardiac causes. Thus, the prognosis in HFPEF depends on cardiac and non-cardiac limitations [3]. Impaired exercise tolerance is a leading clinical feature of those patients. It is currently considered to be associated with the important pathophysiological mechanisms of diastolic dysfunction and LV stiffness [4–6]. LV stiffness can be increased due to microangiopathy, myocyte dysfunction and an increase in fibrosis [3]. Diastolic dysfunction is also associated with reduced cardiac energetic reserve [7] and since iron has an essential role in oxygen metabolism and cellular

Abbreviations: β, constant of LV stiffness, exponential curve fit to EDPVR; CO, cardiac output; dP/dt_{max}, maximum rate of LV pressure change; dP/dt_{min}, minimum rate of LV pressure change; E/A, ratio of early peak (E) to late peak (A) mitral flow velocities; E/E′, LV filling index; E′/A′, early (E′) to late (A′) diastolic velocity ratio of mitral annulus; EDV, end-diastolic volume; EDPVR, end-diastolic pressure–volume relationship; EF, ejection fraction; ESP, end-systolic pressure; ESV, end-systolic volume; ET, exercise testing; FID, functional iron deficiency; HFPEF, heart failure with preserved ejection fraction; IVRT, isovolumic relaxation time; LA, left atrial; LAVI, left atrial volume index; LV, left ventricle; LVEDP, LV end-diastolic pressure; LVMI, LV mass index; PV, pressure volume; S′, systolic velocity of mitral annulus; SW, stroke work; Tau, isovolumic relaxation time constant; TDI, tissue Doppler imaging; VCO2, peak carbon dioxide output; VE, ventilation equivalent; VO2, peak oxygen uptake; VT, ventilatory (anaerobic) threshold.

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energetics, the maintenance of normal iron metabolism is important [8,9]. Anemia is a common comorbidity in patients with heart failure [10]. Its prevalence is found to be similar in patients with preserved and reduced EF, which are 27% and 25%, respectively [11]. Moreover, anemia is an independent predictor of death or hospitalization for cardiovascular reasons among elderly patients with chronic heart failure and reduced or preserved LVEF [12–14]. Beyond impaired erythropoiesis, functional iron deficiency without anemia (FID) also has multifaceted clinical consequences related to the impairment of oxidative metabolism and cellular energetics which is accompanied by reduced aerobic exercise performance [15,16]. It has been recently demonstrated that FID is common and associated with poor outcomes, including reduced exercise capacity, impaired quality of life, and increased hospitalization, in patients with chronic heart failure [17,18]. A large study [19] which used currently accepted criteria for FID (ferritin <100 μg/l, or ferritin of 100–299 μg/l in combination with transferrin saturation <20%) showed that FID was prevalent in up to 37% of the patients with heart failure and reduced EF (HFREF), and independent of the presence of anemia [19,20]. However, the role of FID in patients with HFPEF is still a matter of investigation. Therefore, we aimed to investigate the impact of FID and cardiac iron receptor expression on LV hemodynamics in HFPEF and on exercise capacity which are commonly used as precise predictors of survival in heart failure [21,22].

2. Methods

2.1. Patient population

We investigated 26 patients with stabile HFPEF which were admitted to our unit to have an invasive pressure–volume (PV) analysis performed using a conductance catheter system. The reasons for admission were dyspnea, paroxysmal nocturnal dyspnea, and/or exercise intolerance despite preserved LVEF. According to the level of iron load, the population was divided into subgroups with or without functional iron deficiency, as defined by concentrations of ferritin <100 μg/l or ferritin of 100–299 μg/l in combination with a transferrin saturation <20%.

In all patients, atrial fibrillation, heart valve disease, significant coronary artery disease, and lung diseases had been excluded by means of electrocardiogram, laboratory values, angiography, and/or echocardiography, chest radiography, and lung function tests. All patients gave written consent for invasive diagnostic procedures. The research protocol was approved by the local institutional review committee.

2.2. PV measurements by the conductance catheter method

Three to five days after the exercise testing investigations, PV measurements by use of the conductance catheter method were performed as previously described [6]. The conductance catheter allows continuous online measurements of LV pressure and volume [23]. A 7F combined pressure-conductance catheter (CD Leycom, Zoetermeer, The Netherlands) was introduced retrogradely into the LV by standard methods and connected to a cardiac function laboratory (CD Leycom) for acquisition of the LV volume and pressure, and ECG. The total LV volume was calibrated with thermodilution and hypertonic saline dilution [24]. Hemodynamic indexes were obtained from steady-state PV loops in sinus rhythm. PV relationships were derived from PV loops recorded during preload reduction by temporary balloon occlusion (NuMED, Hopkinton, NY) of the inferior vena cava. Although it has to be conceded that transient vena cava occlusion can result in short-term alterations in sympathetic tone and LV constraint – which can influence the PV relationship – this technique belongs to an established method comparing LV stiffness in control patients with that in heart failure patients. Cardiac performance was assessed by heart rate, stroke volume, end-diastolic volume, end-systolic volume, cardiac output, and stroke work. Systolic load-dependent LV function was determined by measuring the EF, end-systolic pressure, and maximum rate of pressure change (dP/dtmax). Diastolic load-dependent LV function was assessed by the LV end-diastolic pressure (LVEDP), LV minimal pressure (LVPmin), isovolumetric relaxation time constant (Tau), minimal rate of LV pressure change (dP/dtmin), and maximum rate of LV filling (dV/dtmax). We calculated the average slope of the end-diastolic PV relationship (dP/dV) to determine functional LV chamber stiffness (LV stiffness, b) and the exponential curve fit to the diastolic LV PV points to determine how rapidly stiffness (dP/dV) increases with increasing pressure (LV stiffness constant, β). Thus, the end-diastolic PV relationship was fitted with an exponential relation, $LVEDP = c\ \exp(\beta LVEDV)$, to obtain the chamber stiffness constant, c, and the curve-fitting constant, c, as load-independent indexes of diastolic function. Increased LV stiffness was considered present if β (≥0.015/ml) and/or b (≥0.19 mm Hg/ml) were increased in clinically symptomatic patients despite normal EF, as described previously [18].

2.3. Echocardiography

Three to five hours before the PV-loop measurement had been performed, echocardiography studies were performed by 2 independent investigators who were blinded to all information derived by exercise testing and invasive analyses. Mitral flow velocities were recorded in the apical 4-chamber view with a GE System FiVe or Vivid 7 (GE Healthcare, Chalfont St Giles, UK). The LVEF was calculated from two-dimensional apical images according to the Simpson method. Mitral inflow measurements included peak early (E) and peak late (A) flow velocities, the E/A ratio, the deceleration time of early mitral flow velocity (DT), and the isovolumic relaxation time (IVRT). Chamber dimensions were evaluated using standard procedures, including LV mass index and left atrial (LA) diameter. The TDI of the mitral annulus movement was obtained from the apical 4-chamber view. A 1.5-mm sample volume was placed sequentially at the lateral and septal annular sites [25]. The analysis was performed for the systolic (S′) and the early (E′) and late (A′) diastolic peak tissue velocities. The ratio of early to late annular velocity (E′/A′) was determined as a parameter of diastolic function, as well as the LV filling index, by the ratio of transmitral flow velocity to annular velocity (E/E′) as a non-invasive parameter for LV stiffness [26]. Adequate mitral and TDI signals were recorded in all patients.

2.4. Exercise testing

Symptom-limited exercise testing (ET) was performed in all patients using individualized cycle ergometry ramping protocols. β-Blockers, ACE-Inhibitors, angiotensin receptor blockers, calcium channel blockers, and diuretics were withdrawn from the patient's medication 24 h before examination. ET protocols were tailored to yield exercise duration of 8 to 12 min. Heart rate was measured continuously. Standard 12-lead electrocardiograms were obtained at rest, each minute during exercise, and for at least three minutes during the recovery phase. Blood pressure was measured using a standard cuff sphygmomanometer. Ventilation equivalent for oxygen uptake (VEO2) and carbon dioxide output (VECO2) at rest and at ventilatory threshold (VEO2 at VT, VECO2 at VT), peak oxygen uptake at ventilatory threshold (VO2 at VT) and at peak exercise (VO2, [l/min]), peak oxygen uptake per exercise level, breathing reserve, and breathing frequency were ascertained. Peak VO2 was defined as the highest continuous 30-second average VO2 occurring within the final minute of exercise. Peak oxygen consumption (VO2, [ml/min/kg]), was acquired by dividing peak oxygen uptake by the patient's body weight. In this study, the ventilatory threshold (a point during exercise after which ventilation abruptly increases despite gradual increase in work rate and VO2) was identified using the ventilation equivalent of oxygen method (lowest VECO2 value measured during exercise) [27]. All tests were performed under supervision by a physician; all the results were interpreted by a cardiologist and/or a pneumologist who were blinded to all information derived by invasive and echocardiographic analyses.

2.5. Endomyocardial biopsy, myocardial transferrin receptor expression

In a subgroup of HFPEF patients (14/26) endomyocardial biopsies (EMBs) were taken previously in order to exclude myocardial causes for their cardiac dysfunction and exercise intolerance. All patients signed the agreement consent with permission for further analysis of EMBs.

Myocardial tissue samples were obtained by EMBs in 14 HFPEF patients (8 with FID) and in a separate cohort of 6 non-failing patients without ID. EMBs were obtained from the RV septum using a flexible bioptome (Westmed, St. Ingbert, Germany) via the femoral vein approach. Endomyocardial biopsies were directly embedded in optimal cutting temperature compound (Miles Laboratories, Inc., Elkhart, Indiana) and frozen at −70 °C.

Total RNAs were isolated from endomyocardial biopsies using the Trizol reagent (Invitrogen, Karlsruhe, Germany), treated with DNAse (PeqLab, Germany) to remove any traces of genomic DNA and reverse-transcribed to cDNA with the High Capacity Kit (Applied Biosystems, Darmstadt, Germany) using random hexamers.

2.6. Preamplification and gene expression analysis

Differential gene expression was determined by real-time PCR of patients with sufficient biopsy. Preamplification technique was applied due to limited amounts of cDNA [28]. The quantification of myocardial gene expression was performed by real-time RT-PCR reactions containing preamplified cDNA and commercially available TaqMan® gene expression assay for human transferrin receptor (p90, CD71) (Hs00951083_m1, Applied Biosystems, Darmstadt, Germany). Gene expression (E) was normalized to parallel amplified house-keeping gene HPRT applying the formula as described elsewhere [28].

2.7. Statistical analysis

SPSS software for Windows (Version 16.0, SPSS Inc., Chicago, Illinois) was used for statistical analysis. Descriptive characteristics of continuous variables were expressed as median values with the first and third quartiles. Correlation analyses between ET, echocardiographic, PV-loop indexes and iron values were provided using a linear regression model. Comparisons between HPNEF patients with and without iron deficiency were performed with ANOVA if variables were normally distributed, and with the Mann–Whitney *U* test if the data were not normally