Pulmonary Hypertension Is Related to Peripheral Endothelial Dysfunction in Heart Failure With Preserved Ejection Fraction

Farrero et al: Endothelium and Pulmonary Hypertension in HFpEF

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Abstract

Background—Pulmonary hypertension and collagen metabolism abnormalities are prevalent in patients with HFrEF. Peripheral endothelial dysfunction (PED) has been described in heart failure (HF), as well as in pulmonary arterial hypertension. Our aim is to determine whether pulmonary hypertension is associated with PED and impaired collagen metabolism in patients with heart failure and preserved ejection fraction (HFrEF).

Methods and Results—Flow-mediated dilation (FMD) of the brachial artery, matrix metalloproteinase-2 and -9 (MMP-2, -9), tissue metalloproteinase inhibitor 1 (TIMP-1), and C-terminal propeptide of type I procollagen (CICP) were determined in 28 patients with HFrEF and 42 hypertensive controls. Patients with systolic pulmonary artery pressure (PAP)>35 mmHg on echocardiogram underwent a right heart catheterization. HFrEF patients had more severe PED than controls: FMD 1.95%(0.81-4.92) vs. 5.02%(3.90-10.12), p=0.002. Twenty patients with pulmonary hypertension (PH) underwent right heart catheterization: mean PAP 38(27-52)mmHg, wedge capillary pressure 18(16-22)mmHg, pulmonary vascular resistance (PVR) 362(235-603)dyn*s*cm-5. There was a significant inverse correlation between FMD and PVR in HFrEF patients with PH (r=-0.679, p=0.002). HFrEF patients showed higher MMP-2 and CICP values than hypertensive controls. HFrEF patients with higher CICP values also had higher mean PAP (r=0.553, p=0.014), transpulmonary gradient (r=0.560, p=0.013) and pulmonary vascular resistance (r=0.626, p=0.004).

Conclusions—In patients with HFrEF, there is a significant correlation between PED and PVR. Collagen metabolism was more impaired in patients with HFrEF and PH. PED and collagen metabolism assessment could be useful tools to identify HFrEF patients at risk of developing pulmonary hypertension.

Key Words: heart failure and preserved ejection fraction, pulmonary circulation, pulmonary vascular resistance, endothelium dependent dilation, collagen metabolism.
Heart failure with preserved ejection fraction (HFpEF) is an increasingly common diagnosis, accounting for 50% of heart failure (HF) cases (1). Several studies have described the epidemiologic characteristics of the HFpEF population: a predominance of older age, females, and history of hypertension and metabolic syndrome (2). However, the precise mechanism underlying HFpEF is still not well known. The lack of understanding of the physiopathological pathways that lead to the disease may have contributed to the difficulty in finding specific treatments. Several therapies have been assayed with disappointing results and to date there are no evidence-based therapeutic guidelines for this population (3).

Diastolic dysfunction and vascular stiffness have been described in this population and related to an imbalance in extracellular matrix collagen metabolism (4). Peripheral endothelial dysfunction (PED) has been reported in patients with HF (5) and associated with poor outcomes (6). Recent studies report a high prevalence of pulmonary hypertension (PH) in HFpEF, which is in turn related to a worse prognosis (7), although the mechanisms underlying the high prevalence of PH are unknown. Classical studies suggest that increased pulmonary vascular resistance (PVR) is related to abnormalities in smooth muscle tone caused by pulmonary endothelial function as a consequence of NO and endothelin-1 imbalances (8,9). Those imbalances may also affect the peripheral vessels endothelium.

Our aim was to analyze the association between PED, collagen metabolism, and PH in patients with HFpEF. Our working hypothesis was that patients with HFpEF have endothelial dysfunction that affects both the pulmonary and peripheral vasculature. A PED assessment could reflect pulmonary endothelial dysfunction and therefore be related to the presence of PH. Abnormalities in endothelial function could account for a vasoreactive component in PH and HFpEF, in addition to the postcapillary contribution. Imbalanced collagen metabolism could be related to endothelial dysfunction and consequently, PH.
To test our hypothesis, we studied a group of HFrEF patients with PH, assessing peripheral endothelial function, collagen metabolism, and invasive pulmonary hemodynamics, and compared the results to a group of asymptomatic controls with systemic hypertension.

**Methods**

**Study population**

Consecutive adult patients with HFrEF referred to our clinic for HF or PH were prospectively enrolled. Inclusion criteria were at least one hospital admission for HF in the previous year, normal left ventricular systolic function (LVEF≥50%), and diagnosis of HFrEF according to current recommendations (3). Exclusion criteria were untreated ischemic heart disease or valvular heart disease; constrictive parameters; restrictive cardiomyopathies; fibroproliferative systemic diseases including systemic sclerosis, renal failure (Creatinine ≥2.5mg/dl), and lung fibrosis; and significant vascular or parenchymal lung disease: thromboembolic lung disease, pulmonary arterial hypertension, and obstructive or restrictive lung disease (First second Forced Expiratory Volume<55%, Forced Vital Capacity60% and/or Total Lung Capacity<60%).

A group of patients with systemic hypertension who had never presented symptoms or signs of HF were prospectively enrolled as controls. Inclusion criteria were asymptomatic adults with at least 5 years’ history of systemic arterial hypertension. Exclusion criteria were the same as those for HFrEF patients.

The institutional Ethics and Research committee of our hospital approved this study. All patients gave written informed consent.

Controls and patients underwent echocardiogram, endothelial function assessment and blood collection on the same day, one month after discharge if they had been hospitalized. Studies were performed in a blind fashion. Patients with PH who consented, underwent right heart
catheterization in the next 2 weeks after non-invasive evaluation. Images and sera were stored for blinded analysis in a second phase.

**Echocardiogram**

Controls and patients underwent echocardiography evaluation using a commercially available ultrasound system (IE33, Philips Medical Systems, Andover, MA). All parameters were measured in three cardiac cycles (5 cycles in subjects with atrial fibrillation (AF)) and averaged. Right and left ventricular dimensions, left atrium diameter and area, and right ventricular function estimated by tricuspid annular plane systolic excursion (TAPSE) were reported. Left ventricular ejection fraction was assessed by the Simpson method from 2-dimensional apical 2- and 4-chamber views. Preserved systolic function was defined as EF ≥50%. Left ventricular diastolic function was assessed with mitral inflow velocities (E, A) and average of septal and lateral mitral annulus early diastolic velocity by tissue Doppler (e’). E/A and E/e’ ratios were reported. Systolic pulmonary artery pressure (PAP) was estimated by Doppler echocardiography from the systolic right ventricular to right atrial pressure gradient, applying the Bernoulli equation to tricuspid regurgitant wave velocity. Right atrial pressure was estimated using the inferior vena cava diameter and inspiratory oscillations (range 5-20 mmHg), which was added to the calculated gradient to estimate systolic PAP. None of the participants had significant right ventricular outflow tract obstruction.

**Right heart catheterization**

HFpEF patients showing systolic PAP ≥35 mmHg on echocardiogram were proposed to undergo a right heart catheterization. The patient was placed in the supine position, in a fasting state, without premedication.

A 7F thermodilution balloon-tipped catheter (Baxter 139F75) was inserted percutaneously into the brachial, jugular, or femoral vein and advanced under fluoroscopy through the right
heart cavities into the pulmonary artery. The pulmonary capillary wedge position was confirmed by the change from the typical pulmonary artery waveform to the typical pulmonary artery wedge pressure waveform on inflation of the balloon catheter. Pressure transducers were balanced against atmospheric pressure, and the zero reference level was 5 cm below the sternal angle. The following measurements were recorded as the mean of 3 consecutive beats in patients on sinus rhythm (5 beats in AF): right atrial pressure, systolic, diastolic and mean PAP, pulmonary artery wedge pressure at end-expiration, cardiac output as determined by the average of three thermal dilution curves. Cardiac cycles with fusion of two consecutive diastolic waves, as a consequence of a short RR interval, were excluded from analysis. The following parameters were calculated: cardiac index as cardiac output divided by corporal surface area, transpulmonary gradient (TPG) as mean PAP minus pulmonary artery wedge pressure, PVR as TPG divided by cardiac output.

Peripheral endothelial function

Controls and patients underwent peripheral endothelial function evaluation using a commercially available ultrasound system (Sonos 5500, Agilent Technologies, Andover, MA, USA). The method has been previously described (10). Briefly, all participants fasted and avoided exercise, stimulants, and medications for at least 6 hours before the test. They were placed in a quiet, darkened, temperature-controlled room, and all measurements were taken at a similar time of day. Their right arm rested comfortably in a cradle support of the imaged artery for at least 10 minutes before the measurements. A pressure cuff was placed 2 cm distal to the elbow crease. A stereotactically adjustable prop holder was used to achieve a steady image throughout the study, and the sample volume of the pulsed wave Doppler was placed in the middle of the artery as a reference marker. Longitudinal images were obtained by high-resolution ultrasound.

The standard 4-step protocol was used:
1) First baseline scan was recorded.

2) Endothelium-dependent vasodilation was assessed: Pressure cuff was inflated up to 300 mmHg for 5 minutes and released, leading to reactive hyperemia. Pulsed wave Doppler signal of brachial artery flow and 2-dimensional images were scanned 55 to 65 seconds after cuff release.

3) Second baseline scan was obtained after 10 minutes rest to allow vessel recovery.

4) Endothelium-independent vasodilation was assessed: 400 μg of sublingual nitroglycerin was administered and a fourth scan was obtained 3 minutes later.

Images were analyzed by 2 independent observers and averaged. Arterial diameters were determined in an end-diastole frame with dedicated software (QLab, Philips Healthcare, Eindhoven, The Netherlands), placing calipers from the trailing edge of the anterior wall interface to the leading edge of the posterior wall interface and averaging 5 cardiac cycles in patients with sinus rhythm and 10 in patients with AF. Peak brachial artery flow velocity was determined with pulse-doppler sampling volume in the vessel lumen midline with software correction for the incident angle, at rest and for the first 15 seconds after forearm cuff release. Shear rate was calculated as 4*peak flow velocity/arterial diameter.

Flow-mediated vasodilation (FMD) was used as an index of endothelium-dependent dilation and was calculated as the maximal absolute and percentage change in brachial artery diameter after reactive hyperemia divided by that obtained from the first baseline scan (steps 1 and 2). Normal threshold for our lab was considered FMD>5%. Nitroglycerin-mediated dilation was used as an index of endothelium-independent vasodilation and was calculated as the maximal absolute and percentage change in brachial artery diameter after nitroglycerin administration (steps 3 and 4). Using this methodology and a nested analysis of variance, interobserver and intraobserver variance for brachial artery diameter measurement has been
reported as 0.00012 (0.02% of total variability) and 0.00075 (0.13% of total variability), respectively (11).

Collection of blood samples and analysis of extracellular matrix proteins

Circulating matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9), tissue metalloproteinase inhibitor 1 (TIMP-1) and C-terminal propeptide of type I procollagen (CICP) levels were measured when patients were stable, at least a month after hospital discharge. Blood was withdrawn from an antecubital vein into non heparinized tubes. It was kept at room temperature for at least 20 minutes to allow clot formation and then centrifuged at 3000 rpm for 15 minutes at 4°C. Immediately after centrifugation, serum samples were aliquoted and stored at -80°C until assay. Commercially available ELISA kits were used for serum quantification, and their minimum analytical detection limit (DL) were as listed: DMP2F0 for MMP-2 DL=0.047 ng/ml, DMP900 for MMP-9 DL=0.156 ng/ml, DTM100 for TIMP-1 DL=0.08 ng/ml (R&D Systems, Inc. Minneapolis, MN), Microvue 80 for CICP, DL= 0.2ng/ml (Quidel Corporation, San Diego, CA).

Statistical analysis

Participant characteristics are presented as percentage for qualitative variables and as median and quartiles for quantitative variables. Non-parametric tests were used for comparisons throughout the study: Fisher exact test was used to compare qualitative variables and Mann Whitney U test was used to compare quantitative variables. Correlation between plasma biomarkers or FMD and pulmonary hemodynamics was evaluated by linear regression analysis. Since there were extreme values for PVR and TPG, a sensitivity analysis for these variables was performed. All statistical analyses were performed using IBM SPSS 18 software (IBM Corporation, Armonk, NY). Statistical significance was set at two-sided p<0.05.
Results

Population characteristics

Twenty-eight patients with HFpEF were compared to 42 systemic hypertensive controls. Their demographic characteristics are shown in Table 1. As expected, patients with HFpEF had a much higher prevalence of AF (81% vs. 2%, p<0.001) and were more often treated with β-blockers, aldosterone-receptor blockers, diuretics, insulin, vitamin K antagonists, and digoxin. The BNP levels were higher in HFpEF patients than in controls.

Echo parameters

As shown in Table 2, there were no differences between HFpEF patients and hypertensive controls in left ventricular dimensions. Compared to hypertensive controls, patients with HFpEF had a larger right ventricle end-diastolic diameter, a worse right ventricular function (assessed by TAPSE) and signs of a more impaired diastolic function: increased right atrial size, higher E wave velocity, higher E/A ratio and higher E/e' ratio.

The PAP estimated from tricuspid regurgitation jets could be analyzed in 33% of hypertensive controls and in 89% of the HFpEF patients. None of the hypertensive controls and 22 (78%) of the HFpEF patients showed an estimated systolic PAP>35 mmHg.

Pulmonary hemodynamics

Twenty HFpEF patients with PH as determined by echocardiography consented to undergo a right heart catheterization. Their mean PAP was 38(27-52) mmHg, Wedge Capillary Pressure was 18(16-22) mmHg, Cardiac Output 4.3(3.1-5.4) l/min and PVR 362(235-603)dyn*s*cm⁻⁵ (Table 3).

Peripheral endothelial function

Baseline brachial artery diameter did not differ between hypertensive controls and HFpEF patients. There was significantly less FMD in the HFpEF group compared to hypertensive controls, both in absolute and percentage change from baseline diameter (β-coefficient -
0.18mm(-0.28,-0.07), p=0.001 and β-coefficient -4.41%(−7.17, -1.65), p=0.002, respectively). After adjusting for age, sex and nitrate use, differences remained significant (Absolute FMD p=0.001, β-coefficient 0.2mm (0.08-0.32); percentage FMD p=0.001, β-coefficient 5.18%(2.14-8.22)). Nitroglycerin-mediated dilation was similar in both groups (Table 4).

Among the 20 patients with HFpEF and PH who underwent right heart catheterization, subsequent analysis of the association between absolute and percentage FMD and PVR disclosed an inverse correlation (r = -0.679, p = 0.002 and r = -0.623, p = 0.006, respectively); in other words, the less the brachial artery dilated in response to flow, the higher the PVR (Figure 1A).

Absolute and percentage FMD also showed a significant correlation with systolic PAP (r = -0.585, p = 0.011 and r = -0.503, p = 0.033, respectively), diastolic PAP (r = -0.573, p = 0.013 and r = -0.514, p = 0.029, respectively), mean PAP (r = -0.599, p = 0.009 and r = -0.521, p = 0.027, respectively) (Figure 1B), cardiac output (r = 0.520, p = 0.037 and p = 0.479, p = 0.044, respectively) and TPG (r = -0.523, p = 0.026 and r = -0.456, p = 0.057, respectively). One patient showed very high PVR and TPG, and was considered an outlier. After excluding this patient from analysis, the correlation with PVR remained significant (absolute FMD r = -0.586, p = 0.013; percentage FMD r = -0.493, p = 0.044), but not the correlation with TPG. No association was found with pulmonary artery wedge pressure.

**Extracellular matrix proteins**

The HFpEF patients had higher MMP-2 and CICP values than hypertensive controls (β-coefficient 36.09ng/ml(12.26-59.93), p=0.004 and β-coefficient 18.36ng/ml(6.15-30.57), p=0.004, respectively) (Figure 2). After adjusting for age and sex, differences remained significant (MMP-2 β-coefficient 38.14ng/ml(12.83-63.45), p=0.004; CICP β-coefficient 20.43ng/ml(6.68-34.17), p=0.004. There were no differences in MMP-9 (β-coefficient -
87.26ng/ml(-296.44-121.92), p=0.404) or TIMP-1 values (β-coefficient 21.17ng/ml(-15.55-
57.90), p=0.254) between groups.

In the 20 patients who underwent a right heart catheterization, CICP values showed a positive
linear correlation with mean PAP (r=0.513, p=0.029), TPG (r=0.522, p=0.026) and PVR
(r=0.597, p=0.009) (Figure 3). MMP-2, MMP-9, TIMP-1, and BNP levels were not
significantly correlated with pulmonary hemodynamic parameters. One patient showed very
high PVR and TPG, and was considered an outlier. After excluding this patient from analysis,
the correlation with PVR and TPG was not statistically significant.

Participants in the highest tertile of MMP-2 levels had significantly less absolute FMD than
those in the first tertile (0.13(-0.01-0.23)mm vs. 0.22(0.16-0.37)mm, p=0.025). There was a
moderate inverse correlation between MMP-2 or CICP values and lower FMD (r=-0.276,
p=0.034 and r=-0.306, p=0.018, respectively). No association was found between FMD and
MMP-9 or TIMP-1 values.

Discussion
This study shows that patients with HFpEF have impaired peripheral endothelial function
compared to systemic hypertensive patients with diastolic dysfunction who had never
presented HF symptoms. In patients with HFpEF and PH, invasive pulmonary hemodynamics
disclosed a remarkable precapillary component (shown by increased TPG and PVR). In these
patients, we described for the first time an inverse correlation between PVR and peripheral
endothelial function. Patients with increased collagen metabolism proteins showed higher
PAP, TPG and PVR and worse peripheral endothelial dysfunction.

HFpEF is an increasingly prevalent pathology whose underlying mechanisms are not yet
understood. The development of PH in a hypertensive patient with diastolic dysfunction
might be related to the development of HF symptoms. Some studies have reported a
surprisingly high prevalence of PH among HFpEF patients at baseline (7) or during exercise (12) and described an important vasoreactive component (7, 13-15). Moreover, the presence of PH has been associated with an increased mortality in this population (16-19). For these reasons, some authors have studied the potential benefit of pulmonary vasodilators in HFpEF, with controversial results, mainly due to different inclusion criteria related to the presence of PH. (14, 20)

Peripheral endothelial dysfunction

Studies of patients with HF have described PED in the presence of preserved (5) and reduced (21) ejection fraction, as well as pulmonary arterial hypertension (22). The presence of PED has been identified as an independent predictor of cardiovascular events and mortality in the population with HF, HFpEF, and reduced ejection fraction (5, 23), but the mechanism that mediates this association is poorly understood.

Using invasive measurements, we identified an important precapillary component in PH secondary to HFpEF in addition to the postcapillary contribution of pulmonary venous congestion. Our findings indicate a relationship between an impaired peripheral endothelial function and the presence and degree of PH in this subset of patients. This relationship has been described in idiopathic pulmonary arterial hypertension (24), in PH associated with congenital heart disease (25) and in connective tissue diseases such as scleroderma (26), but has never before been reported in HFpEF. The presence of PED may be associated with impaired pulmonary endothelial function and could account for the precapillary component of the PH that has been described in these patients. The worse prognosis of patients with HFpEF and PED compared to patients without PED could be at least partly related to the association between PED and PH.
Extracellular collagen metabolism

Previous studies have shown a progressive increase in extracellular matrix protein circulating levels in healthy controls, hypertensive patients, and HFpEF patients (27, 28). An increased collagen turnover has been linked to a more severe diastolic dysfunction (4) and arterial stiffness (29); therefore, it has been proposed as an etiopathogenic mechanism for HFpEF. Also, high levels of circulating extracellular matrix proteins have been related to the presence of severe pulmonary arterial hypertension (30). Consistent with previous reports, patients with HFpEF in our study showed significantly higher levels of MMP-2 and CICP compared to systemic hypertensive controls. We could also establish a statistical relationship between increased circulating extracellular matrix protein levels, higher PH invasively determined parameters and a more impaired peripheral endothelial function. More studies are needed to assess if collagen metabolism may play a role in the development of endothelial dysfunction and pulmonary hypertension, or if it is just a nonspecific marker of overall HFpEF severity.

Limitations

First, we were able to demonstrate an association between FMD and pulmonary hypertension, but the observational design of the study does not allow us to suggest a causal relationship. Second, the small sample size of our study is due to its invasive nature. However, in contrast to most of the previous studies where indirect measurements are shown, we provide more reliable data. Moreover, our results are consistent with previous literature regarding HFpEF or PH. Third, there were some differences in baseline characteristics between HFpEF and controls regarding gender, medical treatment and AF prevalence. Although peripheral endothelial function measurements were made after 6 hours of medication wash-out, hypertensive controls and HFpEF patients differed in their baseline pharmacological treatment. Spirinolactone, β-blockers, calcium blockers, ACE inhibitors, and ARB have been reported to improve endothelial function (31-34) and were in fact more common in the
HFpEF group; consequently, differences between the groups in peripheral endothelial function could have been underestimated. Fourth, our population had higher PAP and PVR values compared to previous studies (13), which may indicate a selection bias related to the high complexity of our center and the referral of patients with more severe HF. Prevalence of AF was also higher than previously reported for patients with HFpEF, perhaps accounting for the severity (35) of the disease in our cohort. The potential influence of AF on our results must be acknowledged, since pulse irregularity has been reported to be a risk factor for PED independently of HF phenotype (36). It is difficult to discern how much of the differences in FMD are specific to the presence of HFpEF or related to AF. The relationship between FMD and PVR in our patients supports the idea that PED may be related to PH in HFpEF, but the influence of AF in this finding is unclear and should be addressed in future studies.

Conclusion

This study provides evidence that patients with HFpEF have an impaired peripheral endothelial function as compared to hypertensive controls, and that it is associated with the presence of PH and high PVR. Extracellular collagen metabolism abnormalities can be detected in this population. Routine assessment of peripheral endothelial function and extracellular collagen metabolism could help us to identify a subgroup of HFpEF patients at higher risk for the development of PH, and provide a rationale for treating this selected group with pulmonary vasodilators.

Acknowledgments

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Disclosures

None.

References


Table 1. Population characteristics of hypertensive controls (HTN) and patients with HF and preserved ejection fraction (HFpEF)

<table>
<thead>
<tr>
<th></th>
<th>HTN controls (n=42)</th>
<th>HFpEF (n=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>68(61-77)</td>
<td>71(64-78)</td>
<td>0.283</td>
</tr>
<tr>
<td><strong>Female, %</strong></td>
<td>50</td>
<td>82</td>
<td>0.011</td>
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<tr>
<td><strong>Height, cm</strong></td>
<td>1.62(1.58-1.70)</td>
<td>1.58(1.55-1.65)</td>
<td>0.027</td>
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<tr>
<td><strong>Weight, kg</strong></td>
<td>75(69-80)</td>
<td>73(60-84)</td>
<td>0.290</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m2)</strong></td>
<td>27(25-29)</td>
<td>27(24-31)</td>
<td>0.858</td>
</tr>
<tr>
<td><strong>Dyslipidemia, %</strong></td>
<td>38</td>
<td>44</td>
<td>0.624</td>
</tr>
<tr>
<td><strong>Diabetes mellitus, %</strong></td>
<td>28</td>
<td>41</td>
<td>0.310</td>
</tr>
<tr>
<td><strong>Atrial fibrillation, %</strong></td>
<td>2</td>
<td>81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Smoking, %</strong></td>
<td>16</td>
<td>9</td>
<td>0.142</td>
</tr>
<tr>
<td><strong>S-AP, mmHg</strong></td>
<td>134(120-148)</td>
<td>125(110-147)</td>
<td>0.151</td>
</tr>
<tr>
<td><strong>D-AP, mmHg</strong></td>
<td>74(68-83)</td>
<td>64(56-71)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Pulse pressure, mmHg</strong></td>
<td>58(51-71)</td>
<td>66(46-77)</td>
<td>0.605</td>
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<tr>
<td><strong>Heart rate, beats per minute</strong></td>
<td>69(59-79)</td>
<td>70(58-80)</td>
<td>0.704</td>
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<tr>
<td><strong>BNP, pg/mL</strong></td>
<td>44(18-60)</td>
<td>147(82-294)</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Creatinine, mg/dL</strong></td>
<td>0.83(0.70-0.95)</td>
<td>1.12(0.82-1.32)</td>
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<td><strong>GRF, ml/min/m²</strong></td>
<td>36(31-38)</td>
<td>29(25-36)</td>
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<td><strong>Na⁺, mmol/L</strong></td>
<td>141(138-142)</td>
<td>141(139-143)</td>
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<td><strong>Hemoglobin, g/dL</strong></td>
<td>131(116-137)</td>
<td>124(105-133)</td>
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<td><strong>Treatment</strong></td>
<td></td>
<td></td>
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<tr>
<td>β-blocker, %</td>
<td>12</td>
<td>52</td>
<td>0.001</td>
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<tr>
<td>Calcium antagonist, %</td>
<td>19</td>
<td>26</td>
<td>0.562</td>
</tr>
<tr>
<td>ACE-Inhibitor/ARB %</td>
<td>83</td>
<td>74</td>
<td>0.546</td>
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<tr>
<td>Drug</td>
<td>Median</td>
<td>Quartiles</td>
<td>p-Value</td>
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<tr>
<td>-----------------------</td>
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<tr>
<td>Diuretic, %</td>
<td>36</td>
<td>85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin, %</td>
<td>0</td>
<td>22</td>
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<tr>
<td>Oral hypoglycemic drugs, %</td>
<td>24</td>
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<td>37</td>
<td>44</td>
<td>0.615</td>
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<tr>
<td>Vitamin K antagonists, %</td>
<td>2</td>
<td>70</td>
<td>&lt;0.001</td>
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<tr>
<td>Digoxin, %</td>
<td>0</td>
<td>41</td>
<td>&lt;0.001</td>
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<tr>
<td>Nitrates, %</td>
<td>0</td>
<td>22</td>
<td>0.003</td>
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<tr>
<td>Hydralazine, %</td>
<td>0</td>
<td>4</td>
<td>0.397</td>
</tr>
</tbody>
</table>

Values are given as median and quartiles

Abbreviations: ACE-inhibitor, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, brain natriuretic peptide; D-SAP, diastolic systemic arterial pressure; S-SAP, systolic systemic arterial pressure; GFR, Glomerular filtration rate
Table 2. Echocardiographic findings of hypertensive controls (HTN) and patients with heart failure and preserved ejection fraction (HFpEF)

<table>
<thead>
<tr>
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<th>HTN controls (n=42)</th>
<th>HFpEF (n=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejection fraction, %</td>
<td>60(60-65)</td>
<td>58(55-62)</td>
<td>0.016</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>48(45-53)</td>
<td>50(47-54)</td>
<td>0.203</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>30(27-33)</td>
<td>32(28-35)</td>
<td>0.163</td>
</tr>
<tr>
<td>RVEDD, mm</td>
<td>34(30-36)</td>
<td>40(37-44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>12(11-13)</td>
<td>12(12-14)</td>
<td>0.071</td>
</tr>
<tr>
<td>LPW, mm</td>
<td>11(11-12)</td>
<td>12(11-13)</td>
<td>0.093</td>
</tr>
<tr>
<td>Left atrium, mm</td>
<td>38(35-41)</td>
<td>50(42-57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left atrium area, cm²</td>
<td>19 (16-21)</td>
<td>26 (23-33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E-wave velocity, cm/s</td>
<td>61(53-72)</td>
<td>117(80-149)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mitral E/A ratio</td>
<td>0.70(0.59-0.81)</td>
<td>1.9(0.8-3.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tissue Doppler e’ velocity, cm/s</td>
<td>8.3(6.9-10.5)</td>
<td>9.4(8-11.8)</td>
<td>0.089</td>
</tr>
<tr>
<td>Mitral E/e’ ratio</td>
<td>7.1(5.6-9.7)</td>
<td>12.8(9.4-17.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAPSE, mm</td>
<td>23(20-26)</td>
<td>16(13-21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Valid TR jet, %</td>
<td>33%</td>
<td>89%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean estimated S-PAP, mmHg</td>
<td>32(28-34)</td>
<td>62(55-88)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are given as median and quartiles. Abbreviations: IVS, interventricular septum; LPW, left ventricle posterior wall; LVEDD, left ventricle end-diastolic diameter; LVESD, left ventricle end-systolic diameter; RVEDD, right ventricle end-diastolic diameter; S-PAP, systolic pulmonary artery pressure; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation.
Table 3. Pulmonary hemodynamics of patients with heart failure and preserved ejection fraction (HFPEF) and pulmonary hypertension (n=20)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAP, mmHg</td>
<td>12(8-13)</td>
</tr>
<tr>
<td>S-PAP, mmHg</td>
<td>66(59-92)</td>
</tr>
<tr>
<td>D-PAP, mmHg</td>
<td>23(17-32)</td>
</tr>
<tr>
<td>M-PAP, mmHg</td>
<td>38(27-52)</td>
</tr>
<tr>
<td>PAWP, mmHg</td>
<td>18(16-22)</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>4.3(3.1-5.4)</td>
</tr>
<tr>
<td>CI, l/min/m²</td>
<td>2.3(1.9-3)</td>
</tr>
<tr>
<td>TPG, mmHg</td>
<td>18.5(13-30.7)</td>
</tr>
<tr>
<td>PVR, dyn<em>s</em>cm⁻⁵</td>
<td>362(215-603)</td>
</tr>
</tbody>
</table>

Values are given as median and quartiles. Abbreviations: CI, cardiac index; CO, cardiac output; D-PAP, diastolic pulmonary artery pressure; M-PAP, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; S-PAP, systolic pulmonary arterial pressure; TPG, transpulmonary gradient.
Table 4. Peripheral endothelial function of hypertensive controls (HTN) and patients with HF and preserved ejection fraction (HFpEF)

<table>
<thead>
<tr>
<th></th>
<th>HTN controls (n=42)</th>
<th>HFpEF (n=28)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline brachial artery diameter, mm</td>
<td>4.22(3.91-4.86)</td>
<td>4.11(3.63-4.60)</td>
<td>0.634</td>
</tr>
<tr>
<td>Baseline PBFV, cm/s</td>
<td>113(87-123)</td>
<td>108(89-133)</td>
<td>0.763</td>
</tr>
<tr>
<td>Baseline shear rate, s⁻¹</td>
<td>993(788-1208)</td>
<td>1020(796-1430)</td>
<td>0.441</td>
</tr>
<tr>
<td>After cuff occlusion PBFV, cm/s</td>
<td>141(127-170)</td>
<td>136(117-171)</td>
<td>0.769</td>
</tr>
<tr>
<td>After cuff occlusion shear rate, s⁻¹</td>
<td>1235(979-1590)</td>
<td>136(117-171)</td>
<td>0.470</td>
</tr>
<tr>
<td>Absolute FMD, mm</td>
<td>0.21(0.15-0.40)</td>
<td>0.10(-0.03-0.19)</td>
<td>0.001</td>
</tr>
<tr>
<td>Percentage FMD, %</td>
<td>5.02(3.90-10.12)</td>
<td>1.95(-0.81-4.92)</td>
<td>0.002</td>
</tr>
<tr>
<td>Absolute NMD, mm</td>
<td>0.58(0.29-0.67)</td>
<td>0.31(0.19-0.55)</td>
<td>0.066</td>
</tr>
<tr>
<td>Percentage NMD, %</td>
<td>12.52(6.63-15.74)</td>
<td>7.03(3.89-14.23)</td>
<td>0.103</td>
</tr>
</tbody>
</table>

Values are given as median and quartiles

Abbreviations: PBFV, peak blood flow velocity; FMD, flow-mediated dilation; NMD, nitroglycerin-mediated dilation.
Figure Legends

Figure 1. Relationship between peripheral endothelial dysfunction and pulmonary hemodynamics.

Percentage and absolute changes in FMD disclosed an inverse correlation with PVR (1A) and mean PAP (1B), showing that the less the brachial artery dilation in response to flow, the higher the PVR and mean PAP. FMD = Flow-Mediated Dilation; PVR = pulmonary vascular resistance; PAP = pulmonary artery pressure.

Figure 2. Comparison of serum extracellular matrix protein levels.

Patients with HFpEF showed significantly higher circulating levels of CICP and MMP-2 compared to HTN controls. HFpEF = heart failure and preserved ejection fraction; CICP = C-terminal propeptide of type I; MMP-2 = Matrix metalloprotease-2; HTN = hypertensive.

Figure 3. Correlation between serum extracellular matrix proteins and pulmonary hemodynamics.

In patients with heart failure and preserved ejection fraction who had pulmonary hypertension, CICP levels showed a significant positive correlation with mean PAP, TPG and PVR values. CICP = C-terminal propeptide of type I; PAP = pulmonary artery pressure; TPG = transpulmonary gradient; PVR = pulmonary vascular resistance.
Pulmonary Hypertension Is Related to Peripheral Endothelial Dysfunction in Heart Failure With Preserved Ejection Fraction

Marta Farrero, Isabel Blanco, Montserrat Batlle, Evelyn Santiago, Montserrat Cardona, Barbara Vidal, M. Angeles Castel, Marta Sitges, Joan Albert Barbera and Felix Perez-Villa

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