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Filling Pressures and Collagen Metabolism in Hypertensive Patients With Heart Failure and Normal Ejection Fraction

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Abstract—This study was designed to evaluate the association between circulating biomarkers of collagen metabolism and elevated left-sided filling pressures (FPs), as assessed from elevated estimated pulmonary capillary wedge pressure (ePCWP), in hypertensive patients with heart failure with normal ejection fraction. Echocardiography was performed and ePCWP was calculated from the formula ePCWP = 1.90 + 1.24(maximum early transmitral flow velocity in diastole + tissue Doppler early mitral annulus velocity). The biomarkers of collagen synthesis (carboxy-terminal propeptide of procollagen type I) and degradation (matrix metalloproteinase [MMP] 1 and tissue inhibitor of MMP-1 [TIMP-1]) were analyzed by ELISA methods. Seventy-eight patients with normal FPs (ePCWP ≤ 15 mm Hg) and 78 with elevated FPs (ePCWP > 15 mm Hg) were included. Compared with controls, the levels of the 3 biomarkers were increased in the 2 groups of patients. The MMP-1:TIMP-1 ratio, an index of MMP-1 activity, was increased in patients with normal FPs and unchanged in patients with elevated FPs. Patients with elevated FPs exhibited higher TIMP-1 levels and a lower MMP-1:TIMP-1 ratio than patients with normal FPs. ePCWP was independently associated with TIMP-1 (r = 0.349; P < 0.001) and the MMP-1:TIMP-1 ratio (r = −0.240; P < 0.01) in all of the patients. Receiver operating characteristic curves showed that a cutoff value of TIMP-1 of 1557 ng/mL provided 64% sensitivity and 67% specificity for predicting elevated FPs with a relative risk of 3.71 (95% CI: 1.91 to 7.22). These findings suggest that, in hypertensive patients with heart failure with normal ejection fraction and elevated FPs, collagen synthesis predominates over degradation because of a relative excess of TIMP-1. This imbalance can facilitate myocardial fibrosis, which, in turn, may contribute to the elevation of FPs in these patients. (Hypertension. 2010;55:1418-1424.)

Key Words: collagen ■ ejection fraction ■ filling pressures ■ heart failure

More than 50% of heart failure (HF) patients present without a major deficit of left ventricular (LV) systolic function and are presumed to experience HF with normal ejection fraction (HFNEF). The importance of increased left-sided filling pressures (FPs) as a pathophysiological hallmark of HFNEF was reappraised by invasive studies, which showed a uniform presence at rest of increased FPs and which demonstrated increased FPs to limit cardiac performance during atrial pacing and exercise.

Arterial hypertension is frequently associated with HFNEF. It has been shown that myocardial fibrosis, a common finding in postmortem studies and endomyocardial biopsies of patients with hypertensive heart disease, participates in the development of HFNEF in hypertensive patients. In this regard, it has been proposed that fibrosis increases myocardial stiffness, which, in turn, reduces LV compliance during diastole and contributes to elevated FPs in hypertensive patients. However, it is unknown how collagen metabolism is associated with FPs in hypertensive patients with HFNEF.

Recent clinical investigations have allowed the identification of a panel of circulating molecules that fulfil the criteria to be considered as biomarkers of myocardial collagen metabolism in hypertensive patients (reviewed in Reference 8). In particular, serum carboxy-terminal propeptide of procollagen type I (PICP) has been characterized as a biomarker of collagen type I synthesis, whereas the plasma matrix metalloproteinase (MMP) 1: tissue inhibitor of MMP (TIMP)-1 ratio has been shown to be a biomarker of collagen degradation. Although altered levels of serum PICP and plasma TIMP-1 have been reported recently in hypertensive and nonhypertensive patients with HFNEF, no data are available on the relationship of the biomarkers with FPs. Therefore, this study was designed to investigate whether some association exists between elevated FPs and alterations of PICP, MMP-1, TIMP-1, and the MMP-1:TIMP-1 ratio in hypertensive patients with HFNEF.

Methods

Subjects
All of the subjects gave written informed consent to participate in the study, and the institutional review committee approved the

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study protocol. The study conformed to the principles of the Helsinki Declaration. The population was composed of 156 white hypertensive patients with chronic HF. Arterial hypertension was assessed by repeated measures of systolic blood pressure and diastolic blood pressure of >139 and/or 89 mm Hg, respectively. Blood pressure was measured using a mercury sphygmomanometer with an arm cuff appropriate for each subject. Recordings were made in a sitting position and after 5 minutes at rest on 3 occasions, and the mean of these 3 readings was recorded. All of the patients underwent appropriate clinical and laboratory evaluation to exclude secondary hypertension and had a previous clinical diagnosis of chronic HF on the basis of the presence of ≥1 major and 2 minor Framingham criteria, in New York Heart Association (NYHA) functional classes II to IV. None of the patients studied exhibited LV systolic dysfunction, as assessed by an ejection fraction ≤50%. Patients with alcohol abuse, diabetes mellitus, previous myocardial infarction, segmental abnormalities of wall motion, aortic stenosis, mitral regurgitation, hypertrophic cardiomyopathy, absence of sinus rhythm, and previous treatment with cardioactive drugs were excluded. None of the patients presented extracardiac conditions associated with alterations in serum or plasma levels of any of the markers studied (ie, chronic liver disease, renal insufficienty, or metabolic bone disease). Twenty age- and sex-matched healthy, normotensive subjects were used as controls for biochemical parameters.

Echocardiographic Study
2D echocardiographic imaging, targeted M-mode recording, and Doppler ultrasound measurements were obtained in each patient. LV mass was measured, and LV mass index (LVMI) was calculated by dividing LV mass by body surface. The presence of LV hypertrophy was established when LVMI was >122 g/m² for women and >149 g/m² for men. LV end-diastolic volume (LVEDV) was calculated using the Teichholz formula. The following pulsed Doppler measurements of the mitral flow were obtained: maximum early transmural flow velocity in diastole (E), maximum late transmural velocity flow in diastole (A), the deceleration time of the early mitral filling wave (DT), and isovolumic relaxation time (IVRT). Tissue Doppler early mitral annulus velocity (E') was also assessed. The value of E' was averaged from the measurements performed at the septal and lateral annuli. LV endocardial fractional shortening and ejection fraction were calculated according to Quinones et al. LV midwall fractional shortening was calculated according to de Simone et al. FPs were derived from estimated pulmonary capillary wedge pressure (ePCWP). Because an excellent correlation has been observed between the E/E' ratio and invasively assessed pulmonary capillary wedge pressure in patients with a wide range of clinical conditions and rest ejection fractions, ePCWP was calculated using the equation ePCWP = 1.90×1.24 (E/E'). Patients were classified as having normal (ePCWP ≤15 mm Hg) or elevated (ePCWP >15 mm Hg) FPs.

Biochemical Determinations
Venous blood samples were obtained in each patient from the left antecubital vein. PICP was measured in serum by an ELISA method (Metra Biosystems). The interassay and intra-assay coefficients of variation were 11.6% and 5.5% for MMP-1 and 13.1% and 8.9% for TIMP-1, respectively. The lower detection limit was 0.2 ng of PICP per milliliter. MMP-1 and TIMP-1 were measured by ELISA methods (GE Healthcare). The interassay and intra-assay coefficients of variation were 7.2% and 5.5%, respectively, and the lower detection limit was 0.05 ng of MMP-1 per milliliter.

Statistical Analysis
Clinical differences between the 2 groups of patients were analyzed by a Student t test for unpaired data once normality was demonstrated; otherwise, the Mann–Whitney U test was performed. Categorical variables were evaluated by the χ² test. To analyze the differences in biochemical markers of collagen metabolism between control subjects and the 2 groups of patients, 1-way ANOVA followed by a Student-Newman-Keuls test was performed once normality was checked (Shapiro-Wilk test); otherwise, the nonparametric Kruskal-Wallis test followed by a Mann–Whitney U test (adjusting the α-level by Bonferroni inequality) was used. The correlation between continuously distributed variables was tested by correlation coefficients and univariate regression analysis. The influence of confounding factors on correlations was excluded by partial correlation analysis for quantitative parameters and multivariate analysis for qualitative variables. Receiver operating characteristic (ROC) curves allowed determination of the overall performance of TIMP-1 and NT-pro-BNP for identifying elevated FPs in the whole population of patients. Values are expressed as mean±SEM. A value of P<0.05 was considered statistically significant.

Results
Clinical Characteristics
The clinical characteristics of the 2 groups of patients are shown in Table 1. Age was higher in patients with elevated FPs than in patients with normal FPs. Women were more predominant in the group of patients with elevated FPs than in the group of patients with normal FPs. Diastolic blood pressure was lower and pulse pressure higher in patients with elevated FPs than in patients with normal FPs. Although the frequency of patients under treatment with β-blockers was

<p>| Table 1. Clinical Characteristics in the 2 Groups of Patients With HF and Normal Ejection Fraction |</p>
<table>
<thead>
<tr>
<th>Parameters</th>
<th>ePCWP ≤15 mm Hg</th>
<th>ePCWP &gt;15 mm Hg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>72.41±0.95</td>
<td>77.62±0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>47/31</td>
<td>25/53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.97±0.62</td>
<td>31.54±0.57</td>
<td>NS</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>2.21±0.05</td>
<td>2.46±0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>147±2</td>
<td>152±3</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic</td>
<td>87±1</td>
<td>83±2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pulse</td>
<td>60±2</td>
<td>69±3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>79±2</td>
<td>77±2</td>
<td>NS</td>
</tr>
<tr>
<td>Medications, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loop diuretics</td>
<td>76</td>
<td>82</td>
<td>NS</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>51</td>
<td>31</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ACEIs or ARBs</td>
<td>82</td>
<td>81</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>24</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>MR blockers</td>
<td>13</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>NT-pro-BNP, pg/mL</td>
<td>1223±68</td>
<td>1546±93</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

ACEIs indicates angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; MR, mineralocorticoid receptor; NS, nonsignificant. Values are expressed as No. or percentage of subjects and as mean±SEM. The interassay and intra-assay coefficients of variation were 11.6% and 5.5%, respectively, and the lower detection limit was 0.2 ng of PICP per milliliter.
higher in patients with normal FPs than in patients with elevated FPs, no other differences were observed regarding the remaining classes of drugs. Finally, both the severity of HF, as assessed by the NYHA functional class, and plasma NT-pro-BNP were increased in patients with elevated FPs compared with patients with normal FPs.

Table 2 shows the echocardiographic parameters assessed in the 2 groups of patients. The values of both LVMi corrected by LVEDV and relative wall thickness were higher in patients with elevated FPs than in patients with normal FPs. The frequency of LV hypertrophy was higher in patients with elevated FPs than in patients with normal FPs (57% versus 39%; \( P < 0.05 \)). LVEDV corrected by body surface area was reduced in patients with elevated FPs compared with patients with normal FPs. The values of ejection fraction, E, A, deceleration time, the E/E' ratio, and ePCWP were increased in patients with elevated FPs compared with patients with normal FPs.

### Circulating Biomarkers

As shown in Table 3, compared with the control group, serum PICP and MMP-1 levels were increased in the 2 groups of patients, with no significant differences between them. Although serum TIMP-1 levels were higher in the 2 groups of patients than in the control group, the values of this parameter were increased in patients with elevated FPs compared with patients with normal FPs. Compared with controls, the serum MMP-1:TIMP-1 ratio was increased in patients with normal FPs and remained almost unchanged in patients with elevated FPs. In addition, this ratio was decreased in patients with elevated FPs compared with patients with normal FPs.

### Analysis of Associations

Direct correlations were found among ePCWP and PICP \((r = 0.185; P < 0.05)\), TIMP-1 \((r = 0.349; P < 0.001)\); Figure 2), and NT-pro-BNP \((r = 0.180; P < 0.05)\) in all of the patients. In addition, ePCWP was inversely associated with the MMP-1:TIMP-1 ratio \((r = -0.240; P < 0.005)\). In addition, these associations were independent of sex and therapy with \( \beta \)-blockers \((P < 0.01)\).

A direct correlation was found between NT-pro-BNP and TIMP-1 \((r = 0.185; P < 0.05)\); Figure 4). The correlation of NT-pro-BNP with TIMP-1 was independent of age \((r = 0.179; P < 0.05)\), NYHA functional class \((r = 0.199; P < 0.02)\), and pulse pressure \((r = 0.244; P < 0.005)\). In addition, the correlation of ePCWP with the MMP-1:TIMP-1 ratio was independent of age \((r = -0.163; P < 0.05)\), NYHA functional class \((r = -0.178; P < 0.05)\), and pulse pressure \((r = -0.226; P < 0.01)\). In addition, these associations were independent of sex and therapy with \( \beta \)-blockers \((P < 0.01)\).
Analysis of ROC Curves
The ROC curves allowed for determination of the overall performance of TIMP-1 and NT-pro-BNP for identifying elevated FPs in hypertensive patients with HFNEF. The area under the ROC curve was similar for TIMP-1 (0.68±0.04) and for NT-pro-BNP (0.62±0.04), and both curves were higher (P<0.001 and P<0.05, respectively) than 0.50. From the ROC curves, cutoff values of reference for TIMP-1 and NT-pro-BNP were calculated. The sensitivity and specificity of each of these 2 values for identifying elevated FPs are presented in Table 4. Overall, the cutoff value of TIMP-1 showed better specificity and similar sensitivity. The relative risk of presenting elevated FPs was higher for patients with TIMP-1 values >1557 ng/mL than for patients with NT-pro-BNP values >1604 pg/mL (Table 4).

Discussion
The main findings of this study are as follows: (1) serum PICP and MMP-1 are similarly increased in hypertensive patients with HFNEF and elevated FPs and in patients with normal FPs; (2) serum TIMP-1 is more increased in patients with elevated FPs than in patients with normal FPs; (3) the MMP-1:TIMP-1 ratio is increased in patients with normal FPs but is normal in patients with elevated FPs; and (4) serum TIMP-1 is more accurate than NT-pro-BNP in the discrimination of elevated FPs in these patients.

It has been observed that, in the setting of steady-state production by extracardiac sources, serum PICP, MMP-1, and TIMP-1 detected in peripheral blood from hypertensive patients are mainly of cardiac origin, because positive gradients exist for their levels from the coronary sinus toward antecubital vein, and their peripheral and coronary levels are highly correlated (reviewed in Reference 8). In addition, it has been demonstrated in hypertensive patients that serum PICP is associated with the myocardial activation of the enzyme responsible of its formation from its procollagen type I precursor and that the serum MMP-1:TIMP-1 ratio is associated with the degradation and loss of endomysial and perimysial collagen (reviewed in Reference 8). Therefore, the available evidence supports that serum PICP and the MMP-1:TIMP-1 ratio can be considered as biomarkers of myocardial collagen type I synthesis and degradation, respectively, in hypertensive patients.

In this conceptual framework, 2 patterns of myocardial collagen metabolism can be actually distinguished in hypertensive patients with HFNEF from this study: patients with
Indeed, the elevated stiffness in patients with HFNEF is not limited to the myocardium. For instance, intrinsic cardiomyocyte stiffness is significantly increased in these patients. Other mechanisms may also contribute to increased stiffness in HFNEF. For instance, intrinsic cardiomyocyte stiffness is increased in patients with HFNEF. Several arguments support this possibility. First, TIMP-1 binds to the active site of MMP-1 in a 1:1 molar ratio and thereby prevents access to substrates. Thus, the reduced MMP-1:TIMP-1 ratio present in patients with elevated FPs compared with patients with normal FPs would suggest that the activity of MMP-1 is lower in the former than in the latter. Second, TIMP-1 may exert profibrotic actions that appear to be independent of any MMP-1 inhibitory effect. For instance, TIMP-1 may stimulate growth and inhibit apoptosis of fibroblasts, as well as induce the phenotypic differentiation of these cells into a myofibroblast phenotype. Third, as shown by the ROC curve analysis, serum TIMP-1 is a sensitive and specific parameter in the identification of elevated FPs. Fourth, patients with serum concentrations of TIMP-1 >1557 ng/mL have an ~4-fold higher probability of presenting elevated FPs than subjects with serum TIMP-1 below this value. Fifth, serum TIMP-1 presents a better performance for estimating elevated FPs than NT-pro-BNP.

The question emerges as to which factor(s) can be involved in the additional increase of TIMP-1 seen in patients with elevated FPs compared with patients with normal FPs. It is known that TIMP-1 may be induced by several neuropeptides and cytokines activated in HF patients. In particular, it has been reported that BNP is involved in the synthesis of TIMP-1 induced by hypoxia in cardiac fibroblasts. In addition, it has been reported that circulating brain natriuretic peptide and TIMP-1 are correlated in patients with HF. It can thus be speculated that the excess of NT-pro-BNP present in patients with elevated FPs compared with patients with normal FPs may be related to the increase in TIMP-1 seen in the former compared with the latter. In support of this possibility, we found that an independent association exists between NT-pro-BNP and TIMP-1 in the patients from this study.

Several limitations need to be acknowledged. First, this was a study involving a relatively small number of hypertensive patients, in whom the severity and duration of hypertension were not documented, but because of the nature of the goals under investigation, this design is appropriate. Second, although our patient sample was composed of hypertensive patients in whom several cardiac conditions had been excluded, our results may not be generalized to nonhypertensive patients with these cardiac conditions. Third, it must be recognized that therapy may have confounded the findings and their interpretation. Nevertheless, it has been reported that treatment with β-blockers does not modify the levels of the analyzed biomarkers of collagen metabolism. On the other hand, the frequencies of medications that have been demonstrated to influence collagen metabolism in hypertensive patients (eg, the loop diuretic torasemide, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, and mineralocorticoid receptor blockers), were similar in the 2 groups of patients. Fourth, although the measurement

Table 4. Overall Performance of Different Parameters for Predicting Elevated Left-Sided FPs According to ROC curves

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP-1</td>
<td>1557 ng/mL</td>
<td>64</td>
<td>67</td>
<td>15.52</td>
<td>&lt;0.001</td>
<td>3.71</td>
<td>1.91 to 7.22</td>
</tr>
<tr>
<td>NT-pro-BNP</td>
<td>1604 pg/mL</td>
<td>67</td>
<td>50</td>
<td>4.46</td>
<td>&lt;0.05</td>
<td>2.00</td>
<td>1.05 to 3.82</td>
</tr>
</tbody>
</table>

RR indicates relative risk.
of E/E′ can provide misleading information about FPs in patients with advanced HF, recent findings indicate that the measurement remains worthwhile to estimate FPs in patients with HFNEF.²⁸ Fifth, although ePCWP has been shown to be highly correlated with actual pulmonary capillary wedge pressure measured in the catheterization laboratory in HF patients as those included in this study, its load dependence must be recognized.¹⁷ Sixth, although those clinical conditions that may modify circulating levels of the measured biomarkers were carefully excluded in the patients enrolled in the study, we cannot certify that they were in a “steady state” of extracardiac production of the collagen biomarkers measured. Finally, we have not assessed additional circulating biomarkers of collagen metabolism (ie, other collagen-derived peptides and other MMPs and TIMPs), because there is not available evidence showing that the biomarkers actually reflect myocardial collagen metabolism.⁸

**Perspectives**

Our findings suggest that a relationship exists between FPs and the pattern of collagen metabolism in hypertensive patients with HFNEF. Although in patients with normal FPs increased collagen synthesis is associated with increased collagen degradation, in patients with elevated FPs increased collagen synthesis is accompanied by unchanged collagen degradation. This metabolic imbalance may facilitate the development of myocardial fibrosis, which, in turn, may contribute to increased LV stiffness and elevated FPs in these patients. It is likely that improved understanding of the heterogeneity of collagen metabolism in hypertensive patients with HFNEF will facilitate the development of specific therapeutic strategies for better protection of the myocardial structure and the mechanical and functional properties of the left ventricle. In particular, as recently proposed,²⁹ the time is coming to exploit novel, clinically relevant strategies for TIMPs as therapeutic targets in myocardial remodeling. Data on TIMP-1 here provided can be of particular relevance to therapeutic strategies for better protection of the myocardial structure and the mechanical and functional properties of the left ventricle. In particular, as recently proposed,²⁹ the time is coming to exploit novel, clinically relevant strategies for TIMPs as therapeutic targets in myocardial remodeling.

**Acknowledgments**

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**Disclosures**

None.

**References**


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