TIMP-1
A Marker of Left Ventricular Diastolic Dysfunction and Fibrosis in Hypertension

M. Mitchell Lindsay, Paul Maxwell, Francis G. Dunn

Abstract—This study was designed to document noninvasively the pathological mechanisms responsible for myocardial fibrosis and to assess the clinical utility of plasma markers of collagen synthesis and degradation as screening tools for the assessment of fibrosis in hypertension. We studied 100 never-treated hypertensive patients and 50 normal subjects. Echocardiographic assessment was made of left ventricular (LV) mass and diastolic filling using measurement of E:A ratio, E wave deceleration time (E dec), and isovolumic relaxation time (IVRT). The presence of diastolic dysfunction was taken as a surrogate marker for the presence of myocardial fibrosis. Plasma carboxy-terminal propeptide of collagen type I (PICP), carboxy-terminal telopeptide of collagen type I (CITP), and tissue inhibitor of matrix metalloproteinases type I (TIMP-1) were measured as markers of collagen synthesis, degradation, and inhibition of degradation, respectively. Plasma TIMP-1 was significantly elevated in the hypertensive cohort (358 ng/mL versus 253 ng/mL, P<0.001) as were CITP (5.2 μg/L versus 2.9 μg/L, P<0.001), and PICP (200 μg/L versus 166 μg/L, P<0.05). TIMP-1 was significantly elevated in patients with diastolic dysfunction (421 ng/mL versus 283 ng/mL, P<0.01) and correlated with markers of diastolic filling, namely E:A ratio (r=0.26, P<0.05) and E Dec (r=0.41, P<0.01). A plasma TIMP-1 level of >500 ng/mL had a specificity of 97% and a positive predictive value of 96% in predicting diastolic dysfunction. In patients with untreated hypertension, there is evidence of increased collagen synthesis, degradation, and inhibition of degradation resulting in fibrosis. Our results demonstrate that plasma TIMP-1 correlates with markers of LV diastolic filling, is predictive of LV dysfunction, and is a potential noninvasive marker of fibrosis. (Hypertension. 2002;40:136-141.)

Key Words: hypertension, essential ■ hypertrophy ■ diastole ■ collagen ■ fibrosis

Left ventricular hypertrophy (LVH) when defined both by electrocardiogram (ECG) and echo criteria1–3 confers an increased cardiovascular risk in patients with hypertension independent of blood pressure level.4

The 2 key pathological processes in LVH are hypertrophy of the myocyte and fibrosis of the interstitium. The fibrosis results from an increase in the extracellular matrix and, more specifically, an increase in the concentrations of collagen type I and III.5 This is both a primary fibrosis and a secondary reparative fibrosis following myocyte necrosis.6 These findings have been demonstrated in both postmortem studies7,8 and studies using endomyocardial biopsies.9

This accumulation results in a distortion of tissue structure and an increase in myocardial stiffness. This has pathological significance in the development of diastolic dysfunction, as a substrate for ventricular arrhythmias, and ultimately in the development of systolic dysfunction.10

Because of the pathological significance of fibrosis, an understanding of the mechanisms involved and a means of accurately monitoring this process are subjects of consider-
This was achieved firstly by a full cardiac assessment of each patient with ECG, echo, and ambulatory blood pressure studies. Secondly, all patients had a full noninvasive assessment of cardiac fibrosis facilitating a full assessment of the pathological mechanisms responsible for fibrosis in hypertension.

Studies have shown that interstitial fibrosis results in left ventricular (LV) dysfunction, and subsequent regression of this fibrosis with ACE-inhibitors results in improvement in parameters of LV diastolic filling. We therefore took the presence of diastolic dysfunction using conventional echo parameters as noninvasive evidence of interstitial fibrosis in our study.

**Methods**

**Subjects**

The study population consisted of 100 untreated hypertensive patients with hypertension diagnosed on 24-hour blood pressure monitoring (mean 24-hour blood pressure [BP] greater than 140/90 mm Hg). Patients were enrolled via referral to our hypertension clinic or by direct referral from local general practitioners. All patients gave consent and the local research ethics committee approved study protocols.

Exclusion criteria comprised conditions that are known to result in myocardial or tissue fibrosis. These include renal impairment (serum creatinine >130 mmol), coronary artery disease, secondary hypertension, malignancy of any site, pulmonary fibrosis, connective tissue disorders, significant hepatic dysfunction, left ventricular systolic dysfunction, and chronic obstructive pulmonary disease.

The control subjects were enrolled through advertisements in the local press. The normal subjects were determined to be normotensive on 3 standard BP readings, and the above-noted exclusion criteria were applied to this group.

**Study Conditions**

Patients were studied at a standard time in the morning on one study day. A full history and clinical examination was made to identify exclusion criteria and suitability for the study.

**Electrocardiography**

All patients received a standard 12-lead ECG. ECGs were analyzed for the presence of LVH and ST-T changes using standard criteria. Additionally the presence of atrial abnormals was assessed using predefined criteria. All ECGs were analyzed by a single observer (M.M.L.).

**Twenty-Four-Hour BP Monitoring**

Twenty-four-hour BP recordings were taken using a standard oscillometric Spacelab BP monitor (Spacelab 90217). Readings were made every 30 minutes through the daytime period and every hour over the nocturnal period. Patients received at least 1 BP reading per hour, with the requirement that 80% of total readings must be successful and a total of at least 30 readings over the 24-hour period for the blood pressure reading to be accepted. All readings taken during this period were documented, and mean daytime, mean nocturnal, and mean 24-hour blood pressures were calculated. All tapes were analyzed by one observer (M.M.L.).

**Echocardiographic Study**

Patients were studied using a Vingmed CFM800 ultrasound system and a Vingmed System 5 echo machine (both from Vingmed Sound A/S). Examinations were made in a darkened room in the standard left lateral position. Measurements were taken according to the guidelines laid down by the American Society of Echocardiography. Left ventricular mass was calculated using the formula validated by Reichek and Devereux and indexed for body surface area. An average of at least 3 measurements was taken. Measurements of E/A ratio, E wave deceleration time, and isovolumic relaxation time (IVRT) were made in the apical view with a cursor at the mitral valve inflow. An average of 3 measurements was taken at end expiration. A single observer (M.M.L.) made all measurements. Using digital archiving images, intraobserver variability was tested in a blinded fashion. Intraobserver variability was 3.5%, 4%, 10%, and 9% for E/A ratio, E wave deceleration time, IVRT, and calculated left ventricular mass index (LVMI), respectively.

**Biochemical Measurements**

Routine biochemical measurements were taken and analyzed in the standard fashion. All samples were taken at a standard time after 30 minutes in the supine position. Samples were immediately centrifuged and the plasma layer removed. The separated plasma was divided into 3 equal aliquots and frozen at −80°C. Samples were not thawed and refrozen.

We measured plasma P1CP as a marker of collagen synthesis, carboxy-terminal telopeptide of collagen type I (CITP) as a marker of extracellular collagen type 1 degradation, and tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1) as a marker of inhibition of collagen degradation.

Plasma TIMP-1 was measured using a commercially available 2-stage ELISA assay specific for TIMP-1, available from Amersham Pharmaceuticals. All samples were analyzed in duplicate and intra-assay variability was 4.5%.

Plasma CITP was measured by radioimmunoassay using a polyclonal antibody directed against CITP. All samples were run in duplicate with the intra-assay variability calculated as 4.3%.

Plasma P1CP was measured using a radioimmunoassay. Samples were run in duplicate with the calculated intra-assay variability of 4.2%.

Renin and aldosterone were measured using a radioimmunoassay through a routine analyser.

**Statistical Analysis**

The distribution of the collagen markers was tested for normality using the Anderson-Darling test. CITP was normally distributed, whereas both TIMP-1 and P1CP were nonparametric and thus were log-transformed before analysis. These continuous variables were then analyzed using an unpaired 2-tailed Student t test. Data are presented in the nonlogarithmic format. Correlations were tested using the Pearson correlation coefficient in a logarithmic format as required. Noncontinuous variables were analyzed using a χ² test.

**Results**

**Baseline Characteristics**

The baseline characteristics are presented in Table 1. As would be expected, the hypertensive group had a significantly

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hypertensive</th>
<th>Normal</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>100</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>52</td>
<td>51</td>
<td>NS</td>
</tr>
<tr>
<td>Smoker (yes/no/ex)</td>
<td>65/20/13</td>
<td>35/7/8</td>
<td>NS</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>49/51</td>
<td>23/27</td>
<td>NS</td>
</tr>
<tr>
<td>Office systolic BP, mm Hg</td>
<td>167±2.3</td>
<td>129±2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Office diastolic BP, mm Hg</td>
<td>104±1.4</td>
<td>77±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24-h systolic BP, mm Hg</td>
<td>150±1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h diastolic BP, mm Hg</td>
<td>93±0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>5±0.17</td>
<td>5.2±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>96±2.6</td>
<td>97±2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Gamma GT, µ/L</td>
<td>30±3.6</td>
<td>30±5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are mean±SEM. BP indicates blood pressure; Gamma GT, gamma glutamyl transpeptidase; NS, not significant.
higher blood pressure. There was no significant difference in the age, gender distribution, renal function, or hepatic parameters between the 2 groups.

**Electrocardiography**

Using the criteria defined in Methods, 16% of hypertensives and no normals were found to have LVH (P=0.002), 14% and no normals had ST-T changes (P<0.01), 5% had LVH and strain compared with no normals, and 9% had atrial abnormalities compared with 8% of normals in 1 of the 3 criteria used.

**Ambulatory BP**

Readings were on average 90% successful, with a mean total of 40 readings over the 24-hour period. Mean 24-hour BP was 151/93 within this group, with an average 24-hour heart rate of 76 bpm. Analysis of the daytime readings revealed a mean daytime systolic BP of 155 mm Hg and diastolic BP of 97 mm Hg. Daytime heart rate was averaged at 77 bpm. Nocturnal mean systolic BP was 140 mm Hg and diastolic was 84 mm Hg, with a mean nocturnal heart rate of 71 bpm. There was no relationship between BP measurements and collagen markers.

**Echocardiography**

Patients were excluded if poor quality imaging prevented analysis of M-mode and doppler studies. Results are summarized in Table 2. There was a significant increase in the septal (1.14 cm versus 0.9 cm, P<0.001) and posterior wall dimensions (1.18 cm versus 0.9 cm, P=0.004) within the hypertensive group. Calculated left ventricular mass index was also elevated in the hypertensive cohort (132 g/m² versus 97 g/m², P<0.001). All the markers of diastolic filling, ie, IVRT (0.123 s versus 0.099 s, P<0.001), E:A ratio (0.97 versus 1.16, P<0.001), and E wave deceleration times (22.5 ms versus 19.3 ms, P=0.02) were all significantly prolonged in the hypertensive cohort. These measurements reveal that our hypertensive cohort had demonstrable evidence of hypertensive heart disease. The control group had echo parameters within the normal range.

**Collagen Markers**

**TIMP-1**

TIMP-1 levels were significantly elevated in the hypertensive group as a whole compared with normal subjects (385 ng/mL versus 253 ng/mL, P=0.0007; Figure 1). There was no significant difference in TIMP-1 level in the groups with and without LVH (350 ng/mL versus 450 ng/mL, P=NS). Further analysis of the hypertensive group revealed that patients with evidence of diastolic dysfunction, as defined by an E:A ratio <1, had a significantly higher TIMP-1 level than patients with hypertension and normal diastolic filling (421 ng/mL versus 283 ng/mL, P=0.005). Furthermore, there was no significant difference in TIMP-1 levels between normal subjects and hypertensive patients with normal diastolic filling (287 ng/mL versus 253 ng/mL, P=NS; Figure 2). TIMP-1 levels correlated with indices of diastolic filling, namely E:A ratio (r=0.26, P<0.05; Figure 3) and E wave deceleration time (r=0.41, P<0.01; Figure 4). There was no relationship between TIMP-1 levels and indices of left ventricular mass. These findings would suggest that TIMP-1 is a marker of fibrosis and is independent of both blood pressure and markers of left ventricular mass.

In regard to the ECG, TIMP-1 levels were found to be higher in patients with LVH (375 ng/mL versus 337 ng/mL, P=0.05) and patients with ST-T changes (530 ng/mL versus 335 ng/mL, P<0.05). There was no relationship between P1CP or CITP levels and the presence or absence of ECG changes.

**Figure 1.** Data points show plasma TIMP-1 concentrations (ng/mL) in hypertensive patients (HBP) and normal volunteers (Normal) (P<0.001).

**TABLE 2. Echocardiographic Parameters**

<table>
<thead>
<tr>
<th>Echo Parameters</th>
<th>HBP</th>
<th>Normals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSd, cm</td>
<td>1.14±0.02</td>
<td>0.9±0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVPWd, cm</td>
<td>1.18±0.09</td>
<td>0.9±0.02</td>
<td>0.004</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>132±4</td>
<td>97±3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IVRT, s</td>
<td>0.123±0.003</td>
<td>0.099±0.004</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E:A ratio</td>
<td>0.968±0.03</td>
<td>1.16±0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E wave deceleration, ms</td>
<td>22.5±0.8</td>
<td>19.3±0.9</td>
<td>0.02</td>
</tr>
<tr>
<td>% with LVH (LVMI &gt;130 g/m²)</td>
<td>56</td>
<td>2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>% with diastolic dysfunction (E:A ratio &lt;1)</td>
<td>60</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are mean±SEM. IVSd indicates interventricular septal dimension; LVPWd, left ventricular posterior wall dimension; LVMI, left ventricular mass index; IVRT, isovolumic relaxation time; LVH, left ventricular hypertrophy; HBP, hypertensive patients.
To assess whether an elevated TIMP-1 was truly predictive of diastolic dysfunction and, hence, fibrosis, we analyzed hypertensive patients with a TIMP-1/500 ng/mL. We chose this figure because it represented 1 standard deviation above the maximum TIMP-1 level within the normal group. This cohort had an elevated E wave deceleration time and an abnormal E:A ratio, confirming the presence of diastolic dysfunction within this cohort. This was independent of the confounding variables of age, heart rate, and LVMI, thus reflecting a group with genuinely impaired diastolic filling. Indeed, a TIMP-1 level >500 ng/mL in our study showed a specificity of 97% and a positive predictive value in predicting diastolic dysfunction of 96% (Table 3).

Procollagen Type I Carboxy-Terminal Propeptide
Plasma P1CP was elevated in the hypertensive group as a whole compared with normal subjects (200 μg/L versus 166 μg/L, \( P < 0.05 \)). There was no demonstrable relationship between plasma P1CP and any echocardiographic findings or blood pressure parameters.

Carboxy-Terminal Telopeptide of Collagen Type I
Plasma CITP was elevated in the hypertensive group compared with normal subjects (5.2 μg/L versus 2.9 μg/L, \( P < 0.001 \)). There was no relationship between CITP and echocardiographic findings or blood pressure measurements.

Relationships Between Markers
There was no significant relationship seen between TIMP-1 and CITP or P1CP. However, a significant positive correlation was demonstrated between CITP and P1CP, demonstrating increasing collagen degradation in response to increments in collagen synthesis (\( r = 0.35, P < 0.01 \)).

Renin/Aldosterone
Aldosterone levels were significantly elevated in the hypertensive cohort (13.7 ng/100 mL versus 9.3 ng/100 mL, \( P < 0.01 \)). Renin levels were also elevated, but not significantly so. The average aldosterone/renin ratio was 1, and no subject had an elevated ratio consistent with primary aldosteronism. There was no relationship between renin or aldosterone levels with collagen markers or echo parameters.

Discussion
We set out to delineate noninvasively the pathophysiology of fibrosis in hypertensive heart disease and to assess the clinical

<table>
<thead>
<tr>
<th>Variable</th>
<th>Timp-1&gt;500 ng/mL</th>
<th>Timp-1&lt;500 ng/mL</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>52±1.5</td>
<td>53±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>78±1.1</td>
<td>75±1</td>
<td>NS</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>123±3.5</td>
<td>134±4.4</td>
<td>NS</td>
</tr>
<tr>
<td>E:A ratio</td>
<td>0.84±0.03</td>
<td>1±0.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E wave deceleration</td>
<td>27±2.3</td>
<td>20±0.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IVRT</td>
<td>0.13±0.003</td>
<td>0.12±0.003</td>
<td>NS</td>
</tr>
</tbody>
</table>
feasibility of using collagen markers in this setting. This is, by far, the largest study to date in this field. We enrolled a large cohort of well-characterized, never-treated hypertensives. We used robust assays in standard conditions, which we demonstrated to have acceptable reproducibility within our laboratory. It should be accepted that none of the markers used in this study are exclusive to the myocardium. Indeed, previous studies have demonstrated elevation in many fibrotic conditions, including pulmonary fibrosis and hepatic cirrhosis. However, we made strenuous efforts to exclude confounding conditions, enrolling a well-characterized cohort without coexistent conditions leading to fibrosis.

To allow a full assessment of the collagen equilibrium, we measured markers of collagen synthesis, degradation, and inhibition of degradation. We measured PICP as a marker of collagen synthesis. PICP would appear to be the most accurate marker of collagen synthesis because it is reliably cleaved during the extracellular processing of collagen type I, unlike other markers such as procollagen type III amino terminal peptide. Because this is excreted via a hepatobiliary route, patients with hepatic dysfunction were excluded from this study.

Collagen degradation was assessed by measurement of CITP, which is a cross-linked telopeptide released in a 1:1 stoichiometric fashion when collagen type I fibrils are degraded, thus giving an accurate measurement of collagen degradation.

Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases that play a key role in the degradation of collagen. MMPs have potent proteolytic activity, which is controlled in large part by the production of specific naturally occurring inhibitors called tissue inhibitors of matrix metalloproteinases (TIMPs). The best characterized is TIMP-1. Therefore, the measurement of TIMP-1 allows an estimation of the inhibition of degradation.

We demonstrated an elevation in PICP, confirming that collagen synthesis is elevated in hypertensive heart disease. These findings are in agreement with previously published data.

We demonstrated a significant increase in TIMP-1 in the hypertensive cohort taken as a whole. However, only patients with echocardiographic evidence of diastolic dysfunction were found to have a significant elevation in plasma TIMP-1. The remaining patients with normal diastolic filling had TIMP-1 levels comparable to normal subjects. Furthermore, there was no relationship between TIMP-1 and any index of systemic blood pressure or left ventricular mass. Therefore, TIMP-1 levels are not elevated in hypertension per se, but only in patients with diastolic dysfunction and fibrosis. This suggests that the synthesis and release of TIMP-1 is independent of blood pressure and likely to be dependent on a variety of neurohormonal factors. Additionally, we have demonstrated that an elevation in measured TIMP-1 >500 ng/mL is strongly associated with the presence of diastolic function.

One would expect that an elevation in TIMP-1 would result in a reduction in collagen degradation. However, plasma CITP was, in fact, elevated.

Using noninvasive assessment, it is impossible to be sure of the relative roles played by fibrosis/degradation, and there is no doubt that the interplay between TIMP-1 and MMPs is a complex situation. However, we have demonstrated that degradation of collagen is increased in hypertension. Whether this elevation is sufficient to offset the increase in synthesis cannot be determined in this study.

The elevation in TIMP-1 would seem to be at odds with our finding of increased collagen degradation. This need not necessarily be the case. We hypothesize that the elevation in measured plasma TIMP-1, although not sufficient to offset the increment in collagen synthesis, is modulating or limiting collagen degradation and thus contributing to the development of fibrosis.

Our results are at odds with the only previous work published in this field. In that study, Diez et al demonstrated an elevation in TIMP-1 levels and normal CITP levels. This allowed the authors to conclude that collagen degradation is, in fact, inappropriately low. Differences may be explained by the larger numbers in our study, our use of plasma rather than serum (plasma now being regarded as preferable), and our characterization of patients by 24-hour BP rather than solely by office blood pressure.

The other aim of our study was to assess each of the collagen markers as possible noninvasive markers of fibrosis in hypertensive heart disease. Abnormalities in diastolic filling have been shown to precede left ventricular hypertrophy in mild-to-moderate hypertension. In addition, animal and in vivo studies have shown that diastolic dysfunction is predominately secondary to fibrosis and is independent of myocyte hypertrophy. We therefore used diastolic dysfunction as a marker of cardiac fibrosis. CITP and PICP do not appear to be accurate predictors. However, we have shown TIMP-1 to be independent of blood pressure, to correlate with indices of diastolic filling, and to have a specificity and positive predictive value for diastolic dysfunction of over 95%. A measured TIMP-1 >500 ng/mL is an accurate and robust predictor of diastolic dysfunction and, hence, of end-organ damage.

Finally we have demonstrated that TIMP-1 levels were significantly elevated in patients with ECG ST-T changes. This may suggest that ECG ST-T changes in hypertension are indicative of fibrosis.

Perspectives
We have demonstrated, using noninvasive markers of the collagen equilibrium, that untreated essential hypertension is characterized by an increase in collagen synthesis, degradation, and inhibition of degradation. The relative roles played by these processes in the resultant fibrosis cannot be determined by this study. Secondly, we have demonstrated an association between biochemical markers of fibrosis and LV diastolic dysfunction in patients with untreated hypertension. In particular, we identified TIMP-1 as a potential noninvasive marker of fibrosis. Further studies to investigate these markers as predictors of risk are needed and, if positive, will provide an important additional marker in the risk assessment of patients with hypertension.

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