Serum Carboxy-Terminal Propeptide of Procollagen Type I Is a Marker of Myocardial Fibrosis in Hypertensive Heart Disease

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Background—This study was designed to investigate whether the serum concentration of the carboxy-terminal propeptide of procollagen type I (PIP), a marker of collagen type I synthesis, is related to myocardial fibrosis in hypertensive patients.

Methods and Results—The study was performed in 26 patients with essential hypertension in which ischemic cardiomyopathy was excluded after a complete medical workup. Right septal endomyocardial biopsies were performed in hypertensive patients to quantify collagen content. Collagen volume fraction (CVF) was determined on picrosirius red–stained sections with an automated image analysis system. The serum concentration of PIP was measured by specific radioimmunoassay. Compared with normotensives, both serum PIP and CVF were increased ($P<0.001$) in hypertensives. A direct correlation was found between CVF and serum PIP ($r=0.471$, $P<0.02$) in all hypertensives. Histological analysis revealed the presence of 2 subgroups of patients: 8 with severe fibrosis and 18 with nonsevere fibrosis. Serum PIP was higher ($P<0.05$) in patients with severe fibrosis than in patients with nonsevere fibrosis. Using receiver operating characteristic curves, we observed that a cutoff of 127 µg/L for PIP provided 78% specificity and 75% sensitivity for predicting severe fibrosis with a relative risk of 4.80 (95% CI, 1.19 to 19.30).

Conclusions—These results show a strong correlation between myocardial collagen content and the serum concentration of PIP in essential hypertension. Although preliminary, these findings suggest that the determination of PIP may be an easy and reliable method for the screening and diagnosis of severe myocardial fibrosis associated with arterial hypertension. (Circulation. 2000;101:1729-1735.)

Key Words: collagen ■ hypertension ■ myocardium ■ peptides ■ remodeling

Fibrous tissue accumulation is an integral feature of the adverse structural remodeling of cardiac tissue seen in hypertensive heart disease. In fact, an exaggerated accumulation of fibrillar collagens type I and type III occurs throughout the free wall and interventricular septum of animals1–3 and humans4–7 with primary arterial hypertension. A rise in collagen content has been proposed to raise myocardial stiffness and promote abnormalities of cardiac function and electrical activity.8,9 It has been proposed that the excess of myocardial collagen seen in hypertension is the result of both increased collagen synthesis and unchanged or decreased collagen degradation.10

The question arises as to how fibrous tissue should be monitored in hypertensive heart disease. Invasive endomyocardial biopsy is certainly one approach, albeit not widely applicable and manageable. High-frequency backscatter ultrasound is a noninvasive approach that has received attention.11,12 Monitoring of collagen synthesis in serum and other biological fluids has been applied to address tissue repair and collagen turnover in a number of diseases characterized by organ fibrosis.13 More specifically, determinations of serological collagen-derived peptides have been used as markers of fibrillar collagen turnover in various conditions that lead to cardiac fibrosis.14

In spontaneously hypertensive rats (SHR) and patients with essential hypertension, we measured serum concentrations of the carboxy-terminal propeptide of procollagen...
type I (PIP) as a marker of extracellular collagen type I synthesis and the carboxy-terminal telopeptide of collagen type I (CITP) as a marker of extracellular collagen type I degradation. Whereas the serum concentration of PIP was higher in SHR and hypertensive patients than in their normotensive controls, no differences in the serum concentration of CITP were observed between hypertension and normotension either in rats or in humans. In addition, we found a direct correlation between histologically assessed cardiac collagen content and serum PIP in SHR.

These findings allowed us to hypothesize that in arterial hypertension, serum concentration of PIP may be a diagnostic marker of collagen type I-dependent myocardial fibrosis. To definitively test this hypothesis in patients with essential hypertension, the present study was designed to compare PIP with histomorphometric assessment of myocardial fibrosis observed in the interventricular septum tissue obtained by transvenous endomyocardial biopsy. The rationale for the use of this procedure is based on the previous finding that fibrosis present in the septum in postmortem tissue from hypertensive human hearts is representative of fibrosis existing in the free wall.

**Methods**

**Subjects**

The study population consisted of 26 patients (19 men and 7 women; mean age 56 years; range 39 to 70 years) with repeatedly documented elevated systolic blood pressure of >139 mm Hg and diastolic pressure of >89 mm Hg who were referred to our clinic for evaluation and treatment of arterial hypertension. Antihypertensive medication was reported by 16 patients (61%) as monotherapy or in combination. No patient was receiving treatment with either ACE inhibitors or angiotensin II AT1 receptor antagonists, which have been shown to modify serum PIP.

All patients had appropriate clinical and laboratory evaluations to exclude hypertension secondary to renal disorders, renal artery abnormalities, adrenocortical disorders, pheochromocytoma, and iatrogenic causes. All patients had a negative treadmill exercise tolerance test. Selective coronary angiography showed normal epicardial arteries without significant stenoses in all patients. To tolerate test. Selective coronary angiography showed normal abnormalities, adrenocortical disorders, pheochromocytoma, and to exclude hypertension secondary to renal disorders, renal artery.

The study protocol. The study conformed to the principles of the Declaration of Helsinki.

Ten hearts (6 men and 4 women; mean age 59 years, range 40 to 68 years) were collected from a total of 100 autopsies performed at the University Clinic of the University of Navarra during 1998 and 1999. These cases were assumed to represent normal hearts according to the following criteria: (1) sudden death associated with traumatic injury, (2) no medical history or physical and laboratory findings of cardiovascular disease process, (3) absence of clinically recognized systemic disorders, (4) absence of atherosclerosis of the major coronary arteries, and (5) normal cardiac weight. Septal specimens were taken to assess normal values of CVF in these hearts. A group of 24 healthy subjects (15 men and 9 women; mean age 57 years, range 31 to 66 years) who did not undergo the biopsy procedure was also selected to compare PIP with histomorphometric assessment of myocardial fibrosis observed in the interventricular septum tissue obtained by transvenous endomyocardial biopsy. The rationale for the use of this procedure is based on the previous finding that fibrosis present in the septum in postmortem tissue from hypertensive human hearts is representative of fibrosis existing in the free wall.

**Assessment of Left Ventricular Mass and Function**

Two-dimensional, targeted M-mode, and Doppler ultrasound recordings were obtained in each patient as previously described. Left ventricular mass and interventricular septal thickness were measured, and LVMi was calculated by dividing left ventricular mass by body surface area. The following pulsed Doppler measurements were obtained: maximal early transmural velocity in diastole (VE); maximal late transmural velocity in diastole (VA); and IVRT. The ejection fraction was calculated from the measurements performed in a 99mTc ventriculography (mugated acquisition scan, MUGA).

**Determination of Serum PIP**

Serum samples to determine PIP and CITP were taken at the time of clinical studies and stored at −40°C for up to 6 months. Serum PIP was determined by radioimmunoassay according to a method previously described. The interassay and intra-assay variations for determining PIP were 7% and 3%, respectively. The sensitivity (lower detection limit) was 1.20 μg PIP/L.

**Histomorphological Study**

Transvenous endomyocardial biopsies were taken from the middle area of the interventricular septum with a biopomite Cordis 96 cm (7F) under fluoroscopic guidance after angiographic examination. The biopsy procedure was well tolerated. For each patient, 1 to 4 (mean 2.07) biopsy specimens were analyzed. All together, these specimens represented an average endomyocardial space of 2.49 mm² for each patient. Histological evaluation was performed without knowledge of from which patient the tissue section had been obtained.

Myocardial samples were immediately fixed in 10% buffered formalin, embedded in paraffin, and serially sectioned in 4-μm-thick sections. Sections were stained with collagen-specific picrosirius red (Sirius red F3BA in aqueous picric acid) according to Dolber and Spach. CVF was determined by quantitative morphometry with an automated image analysis system (Visilog 4.1.5., Noesis). Sections were analyzed under the microscope (x20), and all the fields covering an endomyocardial area were digitized. Images had a final resolution of 3.37 μm²/pixel (374×276 pixels). Stained collagen areas (dark) were segmented by interactive gray-level thresholding of shading-corrected images, and then the subendocardial regions were interactively discarded. CVF was calculated as the sum of all connective tissue areas divided by the sum of all connective tissue and muscle areas in all the fields analyzed in each section. It has been shown that the total CVF determined by this morphometric approach is closely related to myocardial hydroxyproline concentration. Repeated measurements were performed to assess intraobserver and interobserver variability of histomorphological data. To define the variability of repeated studies, we calculated the coefficient of variation (CV) between the initial and second measurements by use of the SD of differences from measurement of the CVF in the studied patients. The coefficient of error, defined by CV/√n, where n represents the number of measurements, was used to describe the precision of the estimate.

The abnormal accumulation of fibrous tissue was seen as a diffuse increase in red-stained fibers (ie, interstitial fibrosis) and as a localized deposition (ie, perivascular fibrosis and/or microscopic scarring). Three histological grades of interstitial fibrosis were characterized in biopsy tissue: minimal (Figure 1A), mild (Figure 1B), and severe (Figure 1C).

**Statistical Analysis**

Kruskal-Wallis 1-way ANOVA followed by a Mann-Whitney U test (adjusting the α-level by Bonferroni inequality) was used to assess the statistical significance in PIP between normotensives and the 2 subgroups of hypertensives: those with nonsevere fibrosis and those with severe fibrosis. Differences between normotensives and hypertensives and between the 2 subgroups of hypertensives were tested by a Student’s t test for unpaired data once normality was demonstrated (Shapiro-Wilk test); otherwise, a nonparametric test (Mann-
Figure 1. Endomyocardial tissue from 3 hypertensive patients with minimal interstitial fibrosis (A), mild to moderate interstitial fibrosis (B), and severe fibrosis (C). Sections were stained with picrosirius red, and the interstitial collagen was identified in red.
Whitney U test) was used. Categorical variables were analyzed by the χ² test or Fisher’s exact test when necessary. The correlation between continuously distributed variables was tested by univariate and multivariate regression analysis. ROC curves allowed determination of the overall performance of several biochemical (PIP) and echocardiographic (LVMI, VE/V A ) criteria for predicting severe fibrosis in hypertensive patients. Values are expressed as mean±SEM. A value of P<0.05 was considered statistically significant.

Results

Biopsy Data
Collagen volume fraction (CVF) ranged from 1.39% to 2.15% in normal hearts (mean 1.95±0.07%). CVF was out of range in all hypertensive patients but 1, ranging from 2.08% to 9.83% (mean 5.23±0.38%). Thus, CVF was higher (P<0.001) in the myocardium from hypertensive patients than in normal hearts.

Three patients exhibited minimal interstitial fibrosis, 15 patients exhibited mild to moderate interstitial fibrosis, and the remaining 8 patients exhibited severe interstitial fibrosis. Therefore, whereas patients from the first 2 categories were considered to be hypertensives with the nonsevere forms of interstitial fibrosis, patients from the third category were considered to be hypertensives with the severe form of interstitial fibrosis. Perivascular fibrosis was seen in 67% and 31% of patients with severe and nonsevere interstitial fibrosis, respectively. The frequency of microscopic scars was identical (40%) in the 2 subgroups of patients.

As expected, Figure 2 shows that CVF was increased (P<0.05) in hypertensives with severe fibrosis (7.60±0.44%) compared with hypertensives with nonsevere fibrosis (4.08±0.21%) and normal hearts. In addition, CVF was increased (P<0.05) in hypertensives with nonsevere fibrosis compared with normotensives (Figure 2).

Serum PIP
Serum concentration of PIP was higher (P<0.001) in hypertensive patients than in normotensive subjects (118±6 versus 70±5 μg/L). As shown in Figure 3, serum concentration of PIP was higher (P<0.05) in hypertensives with severe fibrosis (140±13 μg/L) than in hypertensives with nonsevere fibrosis (108±6 μg/L) and normotensives. Serum PIP was also increased (P<0.05) in hypertensive patients compared with normotensives (Figure 3).

A direct correlation was found between serum PIP and CVF (r=0.471, P<0.02) in all hypertensives (Figure 4). Multivariate analysis showed that mean arterial pressure and left ventricular mass index (LVMI) enhanced the correlation between PIP and CVF (r=0.649, P<0.01). No significant correlations were found between PIP and other parameters measured in this study.

The receiver operating characteristic (ROC) curves show the overall performance of PIP, LVMI, and the VE/V A ratio for predicting severe myocardial fibrosis (Figure 5). The area under the ROC curve was larger for PIP (0.76±0.10) than for LVMI (0.56±0.13) and for the VE/V A ratio (0.51±0.14). Only the area under the ROC curve for PIP was significantly higher (P<0.05) than 0.50.

According to the ROC curves, the cutoff values of reference for the 3 parameters tested were calculated (Table 1). The sensitivity and specificity of each of these 3 values for predicting severe myocardial fibrosis are presented in Table 1. Overall, the cutoff value of PIP showed the best sensitivity and specificity. Thus, the relative risk of presenting severe myocardial fibrosis was much higher for hypertensive patients with PIP values >127 μg/L than for hypertensive patients with LVMI.
values >122 g/m² or hypertensive patients with VE/VA ratio values <1.02 (Table 1).

**Clinical and Echocardiographic Aspects**
The clinical parameters of the 2 subgroups of hypertensive patients are presented in Table 2. Male patients were predominant in the subgroup with nonsevere fibrosis, but the differences in sex distribution between the 2 subgroups of patients were not statistically significant. Although the duration of hypertension was almost twice as great in hypertensives with severe fibrosis as in hypertensives with nonsevere fibrosis, the differences did not reach statistical significance. No significant differences were observed in the values of blood pressure measured in the 2 subgroups of patients. However, the distribution of the patients in the different stages of arterial hypertension was significantly different (P<0.001), with most hypertensives with nonsevere fibrosis in stage 2 and most hypertensives with severe fibrosis in stage 3. Whereas 44% of hypertensives in the subgroup with nonsevere fibrosis had never been treated, only 25% of hypertensives in the subgroup with severe fibrosis had never been treated.

The calculated LVMI and the interventricular septal thickness were similar in the 2 subgroups of patients (Table 3). The frequency of left ventricular hypertrophy (defined as a LVMI >125 g/m²)26 was 50% in hypertensives with nonsevere fibrosis and 75% in hypertensives with severe fibrosis; this difference was not statistically significant. The values of VE/VA ratio and isovolumic relaxation time (IVRT) were similar in the 2 subgroups of hypertensives (Table 3). The presence of diastolic dysfunction (defined as an altered VE/VA ratio and/or altered IVRT according to age)27 was 61% in hypertensives with nonsevere fibrosis and 62% in hypertensives with severe fibrosis; the difference did not reach statistical significance. Similar values of ejection fraction were measured in the 2 subgroups of hypertensives (Table 3). None of the patients studied exhibited systolic dysfunction.

**Discussion**
The main findings of this study are as follows: (1) a strong association exists between histologically assessed collagen accumulation in biopsied myocardial tissue and serum PIP in patients with essential hypertension, and (2) serum PIP

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**TABLE 1. Overall Performance of Different Parameters for Predicting Hypertensives With Severe Interstitial Fibrosis According to ROC Curves**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cutoff Value</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIP</td>
<td>127 μg/L</td>
<td>75</td>
<td>78</td>
<td>4.80 (1.19–19.30)</td>
</tr>
<tr>
<td>LVMI</td>
<td>122 g/m²</td>
<td>75</td>
<td>44</td>
<td>1.88 (0.47–7.54)</td>
</tr>
<tr>
<td>VE/VA</td>
<td>1.02</td>
<td>50</td>
<td>78</td>
<td>2.25 (0.74–6.81)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM and mean (95% CI).

**TABLE 2. Clinical Parameters Determined in Hypertensive Patients Classified According to the Patterns of Interstitial Fibrosis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>With Nonsevere Fibrosis</th>
<th>With Severe Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54±2</td>
<td>59±4</td>
</tr>
<tr>
<td>Sex, (male/female)</td>
<td>15/3</td>
<td>4/4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.27±1.15</td>
<td>27.30±1.43</td>
</tr>
<tr>
<td>Duration of HBP, y</td>
<td>5.30±1.40</td>
<td>9.54±2.73</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>169±6</td>
<td>182±10</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>99±3</td>
<td>97±4</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>122±3</td>
<td>125±4</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>76±3</td>
<td>71±5</td>
</tr>
<tr>
<td>Stages of arterial hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Stage 2</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Stage 3</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Antihypertensive treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Diuretics</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>β-Receptor blockers</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>α-Receptor blockers</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; HBP, high blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; and HR, heart rate. Values are expressed as mean ± SEM or number of subjects.

**TABLE 3. Echocardiographic Parameters Determined in Hypertensive Patients Classified According to the Patterns of Interstitial Fibrosis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>With Nonsevere Fibrosis</th>
<th>With Severe Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVMI, g/m²</td>
<td>133±7</td>
<td>132±12</td>
</tr>
<tr>
<td>IVST, mm</td>
<td>11.78±0.67</td>
<td>11.32±0.60</td>
</tr>
<tr>
<td>VE/VA</td>
<td>0.96±0.50</td>
<td>0.93±0.13</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>112±4</td>
<td>107±4</td>
</tr>
<tr>
<td>EF, %</td>
<td>61±1</td>
<td>62±3</td>
</tr>
</tbody>
</table>

IVST indicates interventricular septal thickness; EF, ejection fraction. Values are expressed as mean ± SEM.
is more accurate than some echocardiographic parameters in the discrimination of severe myocardial fibrosis from nonsevere myocardial fibrosis in hypertensives.

Pathophysiological Meaning
The rate of extracellular synthesis of collagen type I can be assessed by measuring the serum concentration of PIP, which is freed during the extracellular processing of procollagen type I before collagen molecules form fibers. This peptide is eliminated from the blood by the liver. Taking into account that hepatobiliary function was normal in hypertensive patients studied here, it can be proposed that the abnormally high serum concentration of PIP represents an increased production of the peptide.

A number of observations have led to the proposal that increased production of PIP is a useful marker of stimulated fibrogenesis. Accordingly, the finding of elevated serum concentration of PIP in hypertensives with myocardial fibrosis is in agreement with our previous experimental and clinical findings and reinforces the idea that essential hypertension represents a condition characterized by fibrogenic hyperactivity.

As previously found by others, we did observe that all patients studied showed an excessive collagen accumulation into the myocardium. Furthermore, we found that serum PIP correlates directly with myocardial CVF in hypertensives. Therefore, it is tempting to speculate that increased serum PIP present in patients with essential hypertension may reflect an increased myocardial synthesis of fibrillar collagen type I. This can be of particular relevance in the subgroup of hypertensives characterized by very high concentrations of serum PIP and severe myocardial fibrosis.

Clinical Application
Because myocardial fibrosis is increased in several common types of cardiac disease, including hypertensive heart disease, performing noninvasive characterization of myocardial structure to delineate the extent of collagen accumulation in tissue may play a relevant role in the clinical outcome of these patients.

In this conceptual framework, some findings reported here may be of interest. First, as shown by the ROC curve analysis, serum PIP is a highly sensitive and specific parameter in the identification of severe myocardial fibrosis in hypertension. Second, hypertensives with serum concentrations of PIP >127 μg/L have an almost 5-fold higher probability of presenting with severe myocardial fibrosis than do hypertensives with serum PIP below this value. Third, serum PIP has the highest performance for estimating severe myocardial fibrosis when tested against the standard echocardiographic parameters of left ventricular anatomy (LVMI) and diastolic function (Ve/Va ratio). Therefore, because the determination of serum PIP is simple, reproducible, and low-cost, it may be useful for screening for severe myocardial fibrosis in hypertensive patients, namely in those with stage 3 arterial hypertension.

Our results demonstrate that no association exists between the prevalence of left ventricular hypertrophy and diastolic dysfunction and the extent of myocardial fibrosis in hypertensives. In addition, we found that both LVMI and Ve/Va ratio have a low performance for estimating severe myocardial fibrosis in hypertensives. These findings would suggest that the utility of conventional echocardiographic procedures in the identification of hypertensives with severe forms of myocardial fibrosis is questionable and that the development of alternative methodologies is desirable. In this regard, a correlation between echorectivity and histologically assessed collagen was recently shown in hypertensive patients, suggesting the possibility of noninvasive ultrasonic characterization of myocardial texture in hypertensive heart disease.

Limitations of the Study
Some limitations of the study should be acknowledged. The majority of our patients were under antihypertensive treatment; even though the treatment was inadequate in terms of blood pressure control for all patients, it may have influenced the amount of fibrosis. It is notable that although calcium channel blockers have been shown to prevent myocardial fibrosis in SHR, we did observe that these drugs were more frequently used in the subgroup of hypertensives with severe myocardial fibrosis.

Second, because picrosirius red binds to collagen molecules other than type I, such as type III, and an excess of collagen type III deposition occurs in the left ventricle of patients with essential hypertension, we cannot exclude the possibility that myocardial fibrosis found in the hypertensives studied here is also due to increased deposition of fibril-forming collagen type III molecules.

Finally, it is clear that PIP detectable in serum is not exclusively heart-specific. Nevertheless, we have demonstrated that other extracardiac sources able to elevate serum PIP can be excluded in SHR with increased serum concentration of the peptide. In addition, we have shown that changes in the cardiac compartment of collagen type I alter concentrations of PIP in the circulation of SHR. Whether this is also the case in hypertensive patients deserves further studies.

Conclusions
For the first time, we show that serum levels of PIP correlate with the extent of left ventricular fibrosis in patients with essential hypertension. Thus, the measurement of serum PIP could be practical and useful in the noninvasive assessment of myocardial remodeling in arterial hypertension. In particular, it might have clinical importance in documenting the extent of collagen accumulation and in assessing pharmacological measures designed to prevent its appearance or even to cause its regression. Nevertheless, because of the limitations of this investigation, we are aware that further large studies are necessary to definitively validate this approach.

References


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