Ultrasonic Backscatter and Serum Marker of Cardiac Fibrosis in Hypertensives

Alicia M. Maceira, Joaquin Barba, Nerea Varo, Oscar Beloqui, Javier Díez

Abstract—Elevations in the serum concentration of the carboxy-terminal propeptide of procollagen type I (PIP) >127 µg/L have been found to predict severe myocardial fibrosis in hypertensive patients. This study was designed to assess whether ultrasonic reflectivity, evaluated by a real-time integrated backscatter analysis, was related to the severity of myocardial fibrosis as estimated by serum PIP. Thirty-four subjects were included in the study. Serum PIP was measured by specific radio immunoanalysis. Backscatter cyclic variation and maximal intensity were measured in 6 regions throughout the left ventricle. The subjects were divided into 3 groups: 14 normotensives with PIP <127 µg/L (group 1), 12 hypertensives with PIP <127 µg/L (group 2), and 8 hypertensives with PIP >127 µg/L (group 3). The highest cyclic variation was found in group 1 and the lowest in group 3 (5.78±0.25 versus 4.70±0.33 dB, P<0.05), with intermediate values in group 2 (5.10±0.27 dB). No differences in maximal intensity were found among the 3 groups of subjects. Using receiver operating characteristics curves, we observed that a cutoff of 2.90 dB for cyclic variation measured in the apex provided 75% sensitivity and 63% specificity for predicting PIP >127 µg/L in hypertensives, with a relative risk of 2.50 (95% CI, 0.72 to 34.70). These results show an association between diminished cyclic variation of backscatter and increased serum concentration of PIP in hypertension. Thus, the combination of these 2 parameters may be useful for the diagnosis of severe myocardial fibrosis associated with hypertension. (Hypertension. 2002;39:923-928.)

Key Words: collagen ■ hypertension, essential ■ fibrosis ■ peptides ■ ultrasonography

An exaggerated accumulation of fibrillar collagens type I and type III occurs throughout the free wall and interventricular septum of animals1,2 and humans3,4 with primary arterial hypertension and left ventricular hypertrophy (LVH). A rise in collagen content has been shown to raise myocardial stiffness and promote abnormalities of cardiac function, electrical activity, and intramyocardial perfusion.5

We have used serological markers of collagen turnover to address myocardial fibrosis of rats with spontaneous hypertension (SHR)6–8 and patients with essential hypertension9–12 and LVH. Serum concentrations of the carboxy-terminal propeptide of procollagen type I (PIP), a marker of collagen type I synthesis, were higher in each setting of hypertensive heart disease, and there was a direct correlation between histological evidence and quantity of myocardial fibrosis and serum PIP concentration in SHR and hypertensive patients. Furthermore, in a recent study12 we showed that hypertensives with serum concentrations of PIP >127 µg/L have an almost 5-fold higher probability of presenting with histologically proven severe myocardial fibrosis than do hypertensives with serum PIP below this value. Thus, this cutoff value of PIP can be used to separate hypertensives likely presenting with severe myocardial fibrosis from subjects with nonsevere fibrosis.13

Quantitative characterization of myocardial texture by analysis of ultrasonic reflectivity has been experimentally14–17 and clinically18,19 shown to correlate with the collagen content of the myocardial tissue. We thus have hypothesized that PIP serum concentrations above 127 µg/L should be associated with alterations in myocardial ultrasonic reflectivity detected by backscatter analysis. To test our hypothesis, maximal intensity (MI) and cyclic variation (CV) of backscatter signal were analyzed in normotensive subjects and hypertensive patients classified according to their serum concentrations of PIP.

Methods

Subjects

The study population consisted of 34 subjects (27 men and 7 women, mean age 52 years, range 42 to 68 years) who were referred to our unit for routine cardiac evaluation. Twenty subjects presented elevated systolic blood pressure of >139 mm Hg and diastolic blood pressure of >89 mm Hg. None of the hypertensive patients had been previously treated with antihypertensive medications that would interfere with the renin-angiotensin system. The study was approved
by the review committee of the University Clinic of Navarra and all the subjects gave informed consent.

All patients had appropriate clinical, laboratory, and radiological evaluations to exclude secondary hypertension and cardiac disease different from hypertensive heart disease. None of the subjects exhibited clinical manifestations suggestive of heart failure. Conditions associated with alterations in serum levels of PIP (alcoholic liver disease, metabolic bone disease, hyperthyroidism) were excluded after complete medical examination in all subjects.

**Determination of PIP**

Serum samples for PIP determination were withdrawn at the time of the 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/L.

**Assessment of Left Ventricular Anatomy and Function**

Two-dimensional, targeted M-mode and Doppler ultrasound recordings were obtained in each patient as described previously. Left ventricular mass and interventricular septal thickness (IVST) were measured, and left ventricular mass index (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 (V1), A-wave deceleration time (DT), and isovolumic relaxation time (IVRT). Left ventricular fractional shortening (FS) and ejection fraction (EF) were calculated according to Quinones et al. Diastolic dysfunction was defined as alterations in VE:VA ratio and/or IVST and/or DT according to García et al.

**Analysis of Backscatter Signal**

To perform the backscatter tissue characterization, a SONOS 5500 ultrasound system (Philips) with backscatter software was used. Six regions of interest were chosen throughout the left ventricular myocardium in the following views: mid-septum and mid-posterior wall in the parasternal long axis view, middle portion of the anterior, lateral, and inferior walls in the parasternal short axis view, and apex in the apical 4-chamber view. In each region backscatter images were acquired in continuous loop review format and an independent expert, blinded to the group of pertinence, made the signal analysis off-line. Thus, for each region a time-dependent curve was obtained from which MI and CV were calculated as described elsewhere. For covariates in the statistical analysis, time-gain compensation and depth were also measured in each region.

**Statistical Analysis**

For the statistical analysis, the 10.0 version of SPSS software (SPSS, Inc) was used. All the quantitative variables were tested for Gaussian distribution with the Kolmogorov-Smirnov test; all of them followed this distribution and are presented as mean ± SD.

A repeated-measures mixed factorial design, with myocardial region as intrasubject factor and group of pertinence as intersubject factor, was used. The statistical analysis employed was a 3-way ANOVA (criteria: subjects, group of pertinence, and myocardial segments). Age, sex, intersubject heterogeneity of the signal, LVMI, and echocardiographic time-gain compensation and depth were included as covariates in the analyses. The Bonferroni post-hoc test was used whenever a difference was statistically significant. Receiver operating characteristics (ROC) curves allowed determination of the overall performance of conventional echocardiographic and backscatter measurements for predicting PIP > 127 μg/L in the studied subjects. In all cases a P value of < 0.05 was considered statistically significant.

**Results**

**Clinical Data**

Subjects included in the study were classified in 3 groups according to values of blood pressure and PIP as follows: 14 normotensive subjects with PIP < 127 μg/L (group 1), 12 hypertensive patients with PIP < 127 μg/L (group 2), and 8 hypertensives with PIP > 127 μg/L (group 3).

Clinical parameters evaluated in the 3 groups are presented in Table 1. As expected, values of blood pressure were significantly higher in hypertensives from groups 2 and 3 than in normotensives from group 1. No significant differences in blood pressure were found between the 2 groups of patients. As expected, PIP was significantly higher in hypertensives from group 3 than in the other 2 groups of subjects.

**Left Ventricular Anatomy and Function**

Table 2 shows the echocardiographic parameters assessing left ventricular anatomy and function. The prevalence of LVH was considered to be present when LVMI was higher than 116 g/m² in men and 104 g/m² in women and/or IVST and/or IVRT and/or DT according to García et al.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVMI, g/m²</td>
<td>91 ± 32</td>
<td>119 ± 59</td>
<td>121 ± 36</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>IVST, mm</td>
<td>8.80 ± 2.00</td>
<td>10.80 ± 1.70</td>
<td>10.34 ± 1.70</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>96 ± 18</td>
<td>105 ± 22</td>
<td>101 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>V1/Va</td>
<td>1.10 ± 0.30</td>
<td>1.10 ± 0.30</td>
<td>1.10 ± 0.50</td>
<td>NS</td>
</tr>
<tr>
<td>DT, ms</td>
<td>211 ± 38</td>
<td>218 ± 43</td>
<td>203 ± 57</td>
<td>NS</td>
</tr>
<tr>
<td>FS, %</td>
<td>33 ± 8</td>
<td>31 ± 5</td>
<td>31 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>EF, %</td>
<td>61 ± 6</td>
<td>58 ± 5</td>
<td>59 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>LVH, %</td>
<td>14</td>
<td>54</td>
<td>62</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>DD, %</td>
<td>24</td>
<td>40</td>
<td>64</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Group 1, normotensives with PIP < 127 μg/L; group 2, hypertensives with PIP < 127 μg/L; group 3, hypertensives with PIP > 127 μg/L.

LVMI indicates left ventricular mass index; IVST, interventricular septal thickness; IVRT, isovolumic relaxation time; V1, peak velocity of protodiastolic mitral inflow; Va, peak velocity of end-diastolic mitral inflow; DT, deceleration time; FS, fractional shortening; EF, ejection fraction; LVH, left ventricular hypertrophy; DD, diastolic dysfunction.
LVH was significantly higher in the 2 groups of hypertensives than in the group of normotensives. Similarly, the prevalence of diastolic dysfunction was higher in the 2 groups of hypertensive patients than in the group of normotensive subjects. Furthermore, diastolic dysfunction was more frequent in hypertensives from group 3 than in hypertensives from group 2. None of the subjects studied exhibited systolic dysfunction, as assessed by an EF below 40%.

Backscatter Signal
When we analyzed the CV, time-gain compensation was the only covariate that had a significant impact ($P < 0.05$) and remained in the analysis. CV varied ($P < 0.001$) throughout the myocardial regions, the maximal value being obtained in the inferior and posterior walls and the minimum in the apex in the 3 groups.

As shown in Figure 1, CV was diminished ($P < 0.05$) in hypertensives from group 3 (4.70±0.33 dB, range 4.00 to 5.29 dB) compared with normotensives from group 1 (5.78±0.25 dB, range 5.30 to 6.20 dB). This difference remained significant for all the regions studied (Figure 2). No differences in CV were observed between hypertensives from group 2 (5.10±0.27 dB, range 4.50 to 5.60 dB) and the 2 other groups of subjects.

Regarding MI, the only covariate with a significant impact was, again, time-gain compensation ($P < 0.001$), and this was confirmed in the analysis. MI also varied ($P < 0.001$) among all the myocardial regions in both groups, because it was higher in apex and the anterior wall and lower in the lateral wall. We did not find significant differences in MI among groups, either for global (Figure 3) or regional values (data not shown).

ROC Analysis
The ROC curves assessed the overall performance of conventional and backscatter echocardiographic parameters for predicting values of PIP >127 μg/L. The apical region was chosen to calculate the ROC curve for 2 reasons: (1) backscatter analysis of this region seems to be more accurate because it is not so influenced by attenuation and systolic movement of the heart, and (2) the highest area under the ROC curve was obtained in this region.

The area under the ROC curve was larger ($P < 0.05$) for CV than for the remaining parameters assessed. In addition, only the area under the ROC curve for CV was higher ($P < 0.05$) than 0.50 (Figure 4).

The cutoff values of reference for the different parameters tested were calculated (Table 3) according to the ROC curve. The sensitivity and specificity of each of these values for predicting PIP >127 μg/L are presented in Table 3. Overall, the cutoff value of CV in the apex showed the best sensitivity and specificity. Thus, the relative risk of presenting PIP >127 μg/L was much higher for hypertensives with CV values in the apex below 2.90 dB than for hypertensives with deviations of the cutoff values for the other parameters.

Discussion
The main findings of this study are as follows: (1) an association exists between echocardiographically assessed altered myocardial ultrasonic reflectivity and serum PIP in patients with essential hypertension, and (2) CV of backscat-
ter signal in the apex is more accurate than conventional echocardiographic parameters in the discrimination of increased serum PIP from nonincreased serum PIP.

Several echocardiographic approaches have been used to quantitatively define changes in myocardial ultrasonic reflectivity, among them analysis of the backscatter signal. This signal is produced when the ultrasound interacts with components of the tissue smaller than its wavelength, the reflected signal is scattered, and a part of it (ie, backscatter) is directed toward the probe. Time-domain analysis of this radio frequency signal provides 2 main variables: its maximal intensity or MI and the systolic to diastolic cyclic variation of the intensity or CV. Whereas no significant changes were found in this study in MI, CV was seen to be altered in a group of hypertensive patients characterized by increased serum concentrations of PIP and a high prevalence of LVH. In fact, values of CV measured in hypertensives with PIP above 127 μg/L, were below the interval of normal values measured in this study and reported in the literature.24,25 Several other investigators, using alternative methods, have confirmed the presence of diminished cardiac cycle-dependent variation of backscatter in the hypertrophied myocardium of hypertensive patients.26

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.29 A number of observations have led to the proposal that increased production of PIP is a useful marker of stimulated fibrogenesis in arterial hypertension.33 Furthermore, we have shown recently that a serum concentration of PIP above 127 μg/L is a highly sensitive and specific parameter in the identification of biopsy-proven severe myocardial fibrosis in patients with essential hypertension.11,12 Thus, our finding of decreased CV in hypertensives with elevated PIP would suggest that this ultrasonic abnormality is likely associated with severe myocardial fibrosis in these patients. This possibility is in agreement with previous findings by Ciulla et al26 showing that, in hypertensive patients with LVH and histologically assessed myocardial fibrosis, echo amplitude during cardiac cycle is abnormally diminished.

Because myocardial fibrosis is highly frequent and adversely affects the clinical outcome of hypertensives,3 development of noninvasive tools for its monitoring may play a relevant role in the clinical handling of these patients. In this conceptual framework, some findings reported here may be of interest. First, as shown by the ROC curve analysis, CV in the apex is a highly sensitive and specific parameter in the identification of elevated PIP in hypertension. Second, hypertensives with CV <2.90 dB in the apex have a 2.5-fold higher probability of presenting with elevated PIP than do hypertensives with CV below this value. Third, CV in the apex has the highest performance for estimating elevated PIP when tested against the standard echocardiographic parameters of left ventricular anatomy and function. Therefore, the determination of both CV and PIP may be useful for screening for severe myocardial fibrosis in hypertensive patients.

The mechanism for the reduction in CV of backscatter in hearts from hypertensives with PIP above 127 μg/L is not yet clear. Wickline et al30 showed previously that subepicardial regions in myocardium from open-chest dogs exhibit reduced cyclic variation that parallels their contractile performance. Furthermore, quantitative differences in CV observed among the different myocardial regions have been found to be associated with corresponding regional differences in contractile performance.31 Thus, it is possible that reduced systolic function in hypertensive patients with PIP above 127 μg/L is in part responsible for the observed reduction in CV. The role of myocardial fibrosis in this patient population can be considered as determinant of this functional abnormality. In fact, as proposed by Weber et al,32 whereas a moderate increase in collagen content adversely influences diastolic stiffness of the myocardium and facilitates diastolic dysfunction, a more severe accumulation of collagen fibers is associated with a further rise in diastolic stiffness and a decline in myocardial elastance during contraction, thus leading to systolic dysfunction.

This possibility is in apparent discrepancy with the finding that both subendocardial fractional shortening and ejection fraction were normal in hypertensives with PIP above 127 μg/L. However, it is now accepted that these 2 measures overestimate left ventricular systolic function in most patients with hypertension.33 Other measures provide a different

**TABLE 3. Overall Performance of Different Parameters for Predicting Hypertensives with PIP>127 μg/L**

<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>CV</td>
<td>2.90 dB</td>
<td>75</td>
<td>63</td>
<td>2.50 (0.72–34.70)</td>
</tr>
<tr>
<td>MI</td>
<td>21.65 dB</td>
<td>65</td>
<td>55</td>
<td>1.60 (0.71–7.67)</td>
</tr>
<tr>
<td>LVWI</td>
<td>110 g/m²</td>
<td>57</td>
<td>30</td>
<td>1.67 (0.77–8.22)</td>
</tr>
<tr>
<td>IVRT</td>
<td>95 ms</td>
<td>64</td>
<td>30</td>
<td>1.15 (0.37–4.61)</td>
</tr>
<tr>
<td>V̇e/V̇a</td>
<td>1.14</td>
<td>65</td>
<td>68</td>
<td>1.42 (0.53–6.33)</td>
</tr>
<tr>
<td>DT</td>
<td>205 ms</td>
<td>68</td>
<td>55</td>
<td>1.67 (0.77–8.77)</td>
</tr>
<tr>
<td>FS</td>
<td>30%</td>
<td>64</td>
<td>35</td>
<td>1.33 (0.53–5.57)</td>
</tr>
<tr>
<td>EF, %</td>
<td>58%</td>
<td>60</td>
<td>35</td>
<td>1.02 (0.31–3.31)</td>
</tr>
</tbody>
</table>

CV indicates cyclic variation of backscatter signal in the apex; MI, maximal intensity of backscatter signal in the apex.
impression of the integrity of systolic performance in hypertensives. For instance, midwall shortening has been demonstrated to be impaired in hypertensive patients with normal or greater-than-normal ejection fraction. Thus, assessment of midwall shortening may be useful in detecting compromised systolic function, especially in hypertensives with severe myocardial fibrosis and diminished CV.

Limitations of the Study
Some limitations of the study should be acknowledged. First, it was performed on a limited number of hypertensives. Furthermore, 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 main parameters analyzed.

Second, the specific method of ultrasonic tissue characterization employed here may present some limitations linked to the adjustment of the signal in particular regions of the myocardium. To avoid these problems, we have adjusted the values obtained to local gain and depth in every myocardial region analyzed.

Finally, it is clear that PIP detectable in serum is not exclusively heart-specific. Nevertheless, we have demonstrated that noncardiac sources of elevated serum PIP can be excluded in hypertensive patients 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.

Perspectives
The present study demonstrates that cardiac cycle–dependent variation of backscatter signal is abnormally diminished in hypertensive patients with increased serum concentrations of a marker of severe myocardial fibrosis. Furthermore, the association of PIP above 127 μg/L with CV in the apex below 2.90 dB predicts with great accuracy the presence of severe myocardial fibrosis in arterial hypertension. Thus, the combination of these 2 alterations may be useful for the noninvasive assessment of cardiac fibrosis in hypertensive heart disease. They also set the stage for monitoring this disease in response to pharmacological intervention. Nevertheless, because of the limitations of this investigation, we are aware that further larger studies are necessary to definitively validate this approach.

References


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