Myocardial Ultrasonic Backscatter in Hypertension
Relation to Aldosterone and Endothelin

Michaela Kozáková, Simona Buralli, Carlo Palombo, Giampaolo Bernini, Angelica Moretti, Stefania Favilla, Stefano Taddei, Antonio Salvetti

Abstract—A disproportionate accumulation of fibrillar collagen is a characteristic feature of hypertensive heart disease, but the extent of myocardial fibrosis may differ in different models of hypertension. In experimental studies, aldosterone and endothelins emerge as important determinants of myocardial fibrosis. Changes in myocardial extracellular matrix and collagen deposition can be estimated noninvasively by analysis of the ultrasonic backscatter signal, which arises from tissue heterogeneity within the myocardium and describes myocardial texture. This study was designed to investigate the relations between myocardial integrated backscatter and circulating aldosterone and immunoreactive endothelin in human hypertension. The study population consisted of 56 subjects: 14 healthy normotensive volunteers and 42 hypertensive patients (14 with primary aldosteronism, 7 with renovascular hypertension, and 21 with essential hypertension). The patients with essential and secondary hypertension were matched for age, gender, body mass index, and blood pressure. Myocardial integrated backscatter at diastole was 19.8±2.0 and 20.8±2.9 decibels in normotensive control subjects and patients with essential hypertension and significantly higher in patients with primary aldosteronism (27.4±3.8 decibels, \(P<0.01\)) and renovascular hypertension (26.8±4.8 decibels, \(P<0.01\)). In the population as a whole, as well as in the hypertensive subgroup, myocardial integrated backscatter was directly related to plasma aldosterone \((r=0.73\) and 0.71, \(P<0.01\) for both) and immunoreactive endothelin \((r=0.60\) and 0.56, \(P<0.01\) for both). The data of this study suggest that in human hypertension, circulating aldosterone and immunoreactive endothelin may induce alterations in left ventricular myocardial texture, possibly related to increased myocardial collagen content. (Hypertension. 2003;41:1301–1306.)

Key Words: hypertension, arterial hypertrophy, fibrosis, aldosterone, endothelin, echocardiography

In hypertensive left ventricular (LV) hypertrophy (H), interstitial fibrosis and excessive enlargement of cardiac myocytes occur simultaneously, in varying proportions, depending on the relative weight of blood pressure elevation and specific humoral factors involved.\(^1\)\(^2\)\(^3\) Myocardial fibrosis, characterized by an increase in myocardial collagen content, is supposed to reduce coronary vasodilator capacity and cause LV diastolic dysfunction and heart failure.\(^1\) In experimental studies, aldosterone and endothelins (ET) emerge as important determinants of myocardial fibrosis.\(^4\)\(^5\)\(^6\) Both factors increase the synthesis of collagen I and III by cardiac fibroblasts,\(^4\)\(^5\)\(^6\) and ET-1 also reduces collagenase activity.\(^7\)

The direct pro-fibrotic effect of aldosterone is mediated through specific corticoid receptors in cardiac fibroblasts,\(^8\) and is independent of cardiac load and LVH.\(^9\) In addition, experimental evidence has been collected on a cross-talk between aldosterone and ET. ET-1 has been demonstrated to stimulate aldosterone secretion, both in animals and in humans, having a direct secretagogue effect on the adrenal cortex,\(^10\) equipotent to that of angiotensin II,\(^11\) and aldosterone infusion in experimental models of salt-loaded rats has been shown to enhance ET-1 production.\(^5\)\(^6\)\(^12\)\(^13\)

Ultrasound imaging of the heart has been extensively used to study hypertension-induced changes in LV geometry and function.\(^14\)\(^15\)\(^16\)\(^17\)\(^18\) However, ultrasound signal reflected from the myocardium also contains information on myocardial texture that may be revealed by acoustic tissue characterization. Quantitative texture characterization can be performed through videodensitometric analysis of standard echocardiographic images\(^19\) or through the analysis of ultrasonic backscatter signal. As opposed to standard imaging with strong specular reflection occurring at tissue interfaces (such as epicardium and endocardium), ultrasonic integrated backscatter signal (IBS) is composed of low-amplitude, phasesensitive, omnidirectional echoes arising from tissue heterogeneity within the myocardium.\(^20\) The extracellular matrix has been shown to represent an important source of myocardial IBS,\(^21\) and several experimental studies have demonstrated that IBS in diastole correlates with the collagen content within the myocardium.\(^20\)\(^22\)\(^23\) Therefore, this tech-
nique can be expected to provide noninvasively a relative estimate of myocardial fibrosis in humans. IBS values are highest when the insonifying ultrasound beam is perpendicular to the predominant fiber orientation. Since fiber orientation changes with myocardial contraction, myocardial IBS varies during the cardiac cycle. These cardiac cycle–dependent cyclic variations (CV) of IBS are supposed to reflect the contractile performance of myocardium, although the phenomenon is more complex. In the present study, analysis of the IBS images was performed in hypertensive patients with primary aldosteronism (PA), renovascular hypertension (RVH), and essential hypertension (EH) as well as in normotensive control subjects, with the objective of investigating the relations between myocardial texture, myocardial contractile function, blood pressure, plasma aldosterone, and immunoreactive ET, which is likely to reflect plasma ET-1.

Methods

Study Population

The study population consisted of 56 subjects, including 14 healthy normotensive volunteers (9 men), 21 never-treated patients with EH (14 men), 21 patients with hyperaldosteronism—7 with RVH caused by unilateral renal artery stenosis (5 men; 5 atherosclerotic lesions, 2 fibromuscular dysplasia) and 14 patients with PA (9 men; 6 aldosterone-producing adenoma, 8 bilateral adrenal hyperplasia). Hypertensive patients with EH and hyperaldosteronism were matched for age (51±10 and 48±10 years), gender (67% of men in both groups), body mass index (26.6±2.2 and 27.5±5.3 kg/m^2), and office blood pressure (161±6/100±7 and 163±7/101±7 mm Hg). High blood pressure was defined as an average office blood pressure of 140/90 mm Hg or more on at least two different occasions. EH was diagnosed after exclusion of all possible causes of secondary hypertension. The diagnoses of PA and RVH were made according to standard algorithms, based on clinical history, humoral and electrolyte profiles, and imaging techniques. The study population was free of cardiac or systemic diseases, and the presence of significant coronary artery disease was excluded. None of the patients with EH and 11 recently diagnosed patients with secondary hypertension had ever been treated. The remaining 10 patients with RVH or PA were treated with either diuretic or calcium antagonists or o-blocking agents or a combination of both, until 2 weeks before study, when therapy was discontinued. In PA, the occurrence of hypokalemia was partially corrected by oral potassium supplementation. Patients previously treated by spironolactone, ACE inhibitors, and angiotensin II antagonists had been preliminarily excluded. The institutional review committee approved the study and each participant provided informed consent.

Determination of Humoral Profile

Plasma renin activity, aldosterone, and immunoreactive ET were measured in the morning, after an overnight fast with the subject in the standing position for 1 hour. All subjects were under controlled salt intake (60 to 80 mmol of sodium per day and 60 to 80 mmol of potassium per day). Radioimmunoassays were used to measure plasma renin activity, plasma aldosterone, and immunoreactive ET. In our laboratory, intra-assay and interassay coefficients of variation for immunoreactive ET were 2.9% and 10.5%, respectively, and normal value was up to 1.5 pmol/L. Cross-reactivity for the other peptides was 142% for ET-2, 98% for ET-3, and 0.05 for all others. A spectrophotometric method was used to assess urine hydroxyproline, taken as a rough index of collagen turnover. To avoid interference with the colorimetric method used, all subjects studied received a specific diet without meat, fish, and ice cream for 3 days before urine collection. In our laboratory, intra-assay and interassay coefficients of variation for urine hydroxyproline were 4.5% and 6.3%, respectively.

Echocardiographic Evaluation

Echocardiographic evaluation included (1) standard transthoracic 2-dimensionally targeted M-mode and Doppler echocardiography and (2) acoustic texture characterization.

Transthoracic echocardiograms were performed according to standard protocols, with LV mass and mass index, relative wall thickness, endocardial shortening, and afterload-corrected midwall shortening being calculated according to ASE recommendations with standard formulas. Doppler echocardiography was used to calculate cardiac output and to evaluate transmitral flow velocity profile, where peak early (E wave) flow velocity (PEV), peak late (A wave) flow velocity (PAV), acceleration time of PEV, and PEV and A-wave integrals (Ei and Acriptive), and atrial contribution to LV filling (ACLVF), that is, the percentage of the A-wave area in relation to the total area under the curve of diastolic flow velocity profile, were calculated.

Acoustic tissue characterization was performed at the midportion of the interventricular septum by using an ultrasonic imaging system (SONOS 5500, Philips Technologies) operating in the acoustic densitometry acquisition mode that permits the acquisition, display and analysis of real-time IBS images on the basis of a previously described technique. IBS images of interventricular septum were obtained in the parasternal long-axis view, since in this projection the insonifying ultrasound beam is perpendicular to the predominant fiber orientation. Acquisition of IBS images was performed according to two different protocols: one for assessing myocardial IBS and the other for measuring CV of IBS. In the protocol designed to assess myocardial IBS, time and lateral gain compensations were set off, and the imaging depth and transmit power were set to optimize image quality in the first patient of the study and then left unchanged for all consecutive subjects. In this way, we could ensure that the myocardial IBS values were obtained under comparable conditions in the various study subjects. In the protocol designed to measure cardiac cycle–dependent CV of IBS, transit power and time compensation values were set to optimize image quality in each subject, so that the maximum CV of IBS could be obtained.

Digital sequences (60 consecutive frames) of IBS images were acquired at a rate of 30 frames per second, stored on optical disk, and subsequently analyzed with the acoustic densitometry analysis package. An elliptical region of interest of adequate size (21×21 pixels) was placed in the midportion of the interventricular septum, carefully avoiding the bright endocardial borders. In each frame, the mean integrated backscatter was measured within the region of interest and expressed in decibels. The position of the region of interest was adjusted in each frame so that the same area of interventricular septum was analyzed throughout the digital sequence. In IBS images aimed to assess myocardial IBS, 4 consecutive frames with the highest values of IBS (diastolic frames) were selected from each cardiac cycle, the mean value calculated and averaged across all cardiac cycles during the cineloop (two to three, depending on heart rate). In IBS images aimed to assess CV of IBS, 4 consecutive frames with the highest and 4 with the lowest values of IBS (systolic frames) were selected, and the difference between mean diastolic and mean systolic values, that is, CV of IBS, calculated. CV of IBS was also averaged across all cardiac cycles throughout the cineloop.

Reproducibility of the IBS measurements was tested in 18 patients by assessing diastolic IBS and CV values in two different acquisitions performed and analyzed in the same session by the same operator (intraobserver variability) and by two different operators, blinded each to the other (interobserver variability). Agreement between different acquisitions was evaluated by the consistent bias. The mean difference between the two series of acquisitions performed by the same operator was 0.13±1.3 dB for myocardial IBS and −0.05±0.54 dB for CV of IBS (averaged variation, 5.4% and 9.2%). The mean difference between the two series of acquisitions performed by different operators was −0.67±1.93 dB for myocardial IBS and 0.22±0.67 dB for CV of IBS (averaged variation, 7.2% and 11.4%). Plots of the differences for myocardial IBS and CV values showed all points, or all points but one, within ±2 SD of the mean difference. In addition, long-term consistency of the measurements was tested in 10 normotensive control subjects by...
TABLE 1. Clinical Features, Electrolytes, and Humoral Profile of Normotensive Controls and Patients with Essential Hypertension, Unilateral Renovascular Hypertension, and Primary Aldosteronism

<table>
<thead>
<tr>
<th>Clinical Profile</th>
<th>Controls</th>
<th>EH</th>
<th>RVH</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50 ± 5</td>
<td>51 ± 10</td>
<td>52 ± 10</td>
<td>47 ± 9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.7 ± 2.7</td>
<td>26.6 ± 2.6*</td>
<td>26.2 ± 4.3</td>
<td>28.0 ± 3.1**</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>116 ± 15</td>
<td>161 ± 6**</td>
<td>163 ± 10**</td>
<td>163 ± 4**</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>69 ± 12</td>
<td>100 ± 7**</td>
<td>102 ± 9**</td>
<td>107 ± 7**</td>
</tr>
<tr>
<td>Duration of hypertension, mo</td>
<td>33 ± 13</td>
<td>50 ± 36</td>
<td>46 ± 37</td>
<td></td>
</tr>
<tr>
<td>Plasma potassium, mmol/L</td>
<td>4.2 ± 0.4</td>
<td>4.0 ± 0.2*</td>
<td>3.2 ± 0.2**††</td>
<td>3.0 ± 0.2**††</td>
</tr>
<tr>
<td>Plasma renin activity, ng Angl/mL per hour</td>
<td>1.09 ± 0.58</td>
<td>1.33 ± 0.89</td>
<td>2.55 ± 1.30***</td>
<td>0.16 ± 0.11***†††</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>558 ± 147</td>
<td>589 ± 199</td>
<td>1165 ± 494**††</td>
<td>1292 ± 548**†††</td>
</tr>
<tr>
<td>Immunoreactive endothelin, pmol/L</td>
<td>1.09 ± 0.38</td>
<td>1.22 ± 0.31</td>
<td>1.49 ± 0.27*</td>
<td>2.00 ± 0.59**†††</td>
</tr>
<tr>
<td>Urine hydroxyproline, µmol/L per day</td>
<td>212 ± 31</td>
<td>257 ± 63*</td>
<td>285 ± 44**</td>
<td>309 ± 43**†††</td>
</tr>
<tr>
<td>ECG PQ interval, ms</td>
<td>141 ± 14</td>
<td>149 ± 16</td>
<td>161 ± 19*</td>
<td>176 ± 22**†††</td>
</tr>
</tbody>
</table>

* and **P<0.05 and P<0.01 vs controls; † and ††P<0.05 and P<0.01 vs EH; †††P<0.01 vs unilateral RVH.

Comparing two different acquisitions performed by the same operator at a distance of 3 months. The mean difference between the acquisitions was 0.11 ± 1.53 dB for myocardial IBS and −0.01 ± 0.60 for CV of IBS (averaged variation, 7.2% and 9.4%). Plots of the differences for myocardial IBS and CV values showed all points within ±2 SD of the mean differences.

Data Analysis

Data were expressed as mean ± SD. One-factor ANOVA was used to compare normal control subjects and subgroups of hypertensive patients, followed by the Scheffé post hoc test when needed. When appropriate, ANCOVA was used to adjust for significant group differences. The relations of myocardial IBS and CV of IBS to continuous variables were assessed by least-squares linear regressions. Variables that were significantly related to these dependent variables in univariate analyses and that did not exhibit excessive collinearity with each other were considered potential independent variables in multiple linear regression. Statistical analysis was performed by commonly available software (StatView 5.0, Abacus Concepts Inc).

Results

Clinical Features and Humoral Profile of the Patients

The hypertensive patients had higher body mass index than normotensive control subjects (Table 1). Hypertensive groups were comparable for office systolic and diastolic blood pressure, whereas EH patients had a shorter duration of hypertension. As expected, plasma potassium was decreased and aldosterone increased in patients with PA and RVH. Patients with PA had low and patients with RVH had high plasma renin activity. Compared with normotensive control subjects, plasma immunoreactive ET was increased in patients with PA and RVH and urine hydroxyproline in all hypertensive groups. Patients with PA and RVH also had a longer PQ interval on ECG.

Echocardiographic Findings

Left ventricular mass index and relative wall thickness were higher in patients with PA than in patients with EH (Table 2).
Endocardial shortening and cardiac index were comparable between hypertensive patients and normotensive control subjects, whereas afterload-corrected midwall shortening was lower in all hypertensive groups, although more significantly in patients with PA. Doppler-derived indexes of LV diastolic filling were, to some degree, altered in all the hypertensive groups; however, only E/A ratio and ACLVF significantly differed between patients with EH and patients with hyperaldosteronism. Compared with normotensive control subjects, myocardial IBS was increased in patients with RVH and PA, whereas CV of IBS was decreased in all hypertensive patients, more significantly in those with PA.

In the whole population studied, myocardial IBS was positively related to plasma aldosterone ($r=0.73$, $P<0.01$), immunoreactive ET ($r=0.60$, $P<0.01$), urine hydroxyproline ($r=0.48$, $P<0.01$), LV mass ($r=0.52$, $P<0.01$), interventricular septal thickness ($r=0.54$, $P<0.01$), relative wall thickness ($r=0.46$, $P<0.01$), ACLVF ($r=0.41$, $P<0.01$), and negatively to afterload-corrected midwall shortening ($r=-0.46$, $P<0.01$), acceleration time of PEV ($r=-0.35$, $P<0.05$), and E/A ratio ($r=-0.44$, $P<0.01$) (Figure, A and B). In the hypertensive subpopulation, these relations remained significant (with $r$ values of 0.32 to 0.71, $P$ at least $<0.05$), and myocardial IBS was also related to duration of hypertension ($r=0.38$, $P<0.05$). In the multivariate model, which also took into account blood pressure, plasma aldosterone, interventricular septal thickness, and immunoreactive ET were independently related to myocardial IBS ($F$ value 23.2, 7.7 and 6.5; adjusted $R^2=0.64$). A separate analysis of 16 patients with hyperaldosteronism (11 PA and 5 RVH) and 16 patients with EH, matched for duration of hypertension (30±19 versus 31±15 months) and blood pressure (161±5/101±7 versus 161±6/102±8 mm Hg), was performed to exclude that the difference in the duration of disease could largely count for differences in myocardial IBS between groups. Also in this sub-population myocardial IBS was
significantly higher in patients with hyperaldosteronism (26.3±4.2 dB, P<0.01) than in patients with EH (21.2±2.5 dB).

In the whole population, CV of IBS was negatively related to plasma aldosterone (r=-0.44, P<0.01), urine hydroxyproline (r=-0.38, P<0.01), LV mass (r=-0.27, P<0.05), interventricular septal thickness (r=-0.29, P<0.05), relative wall thickness (r=-0.34, P=0.05), ACLVF (r=-0.48, P<0.01) and positively to afterload-corrected midwall shortening (r=0.34, P<0.05) and E/A, ratio (r=0.50, P<0.01). In the hypertensive population alone, CV of IBS was significantly related to plasma aldosterone, ACLVF, and E/A, ratio (r=-0.39, -0.35, and 0.40, P at least <0.05). In the multivariate model, E/A, ratio and plasma aldosterone remained independently related to CV of IBS (F value 9.7 and 5.2; adjusted R²=0.29).

In the entire study population, aldosterone was also related to LV mass, interventricular septal thickness, afterload-corrected midwall shortening, E/A, ratio, and ACLVF (r=0.37, 0.40, –0.38, –0.38, and 0.36, respectively; P<0.01 for all), whereas immunoreactive ET was related to LV mass, interventricular septal thickness, and E/A, ratio (r=0.30, 0.31, –0.37, respectively; P at least <0.05). With increasing LV mass and septal thickness, urine hydroxyproline also increased (r=0.58 and 0.56, P<0.01 for both).

Discussion

The main findings of this study, achieved in a merged population of normotensive control subjects, patients with EH and hyperaldosteronism, were (1) myocardial IBS increases with plasma aldosterone and immunoreactive ET, which is very likely to reflect plasma ET-1; (2) with increasing myocardial IBS, LV mass and wall thickness increase, afterload-corrected midwall shortening deteriorates, and LV filling pattern exhibits a greater dependence of diastolic filling from the late, presystolic component; (3) CV of IBS and afterload-corrected midwall shortening decrease with increasing plasma aldosterone. These data support the hypothesis that in human heart, aldosterone and ET may contribute to changes in extracellular matrix and suggest that these structural changes result in alterations of myocardial systolic performance and LV diastolic filling.

Myocardial IBS, LV Mass, Aldosterone, and Immunoreactive ET

The present study confirms and extends previous clinical studies that have appraised the possible interrelations between plasma aldosterone, LV remodeling, myocardiastuffness, and myocardial texture.

Rossi et al., in a group of patients with EH and PA, demonstrated that plasma aldosterone was directly related to LV wall thickness and that patients with PA showed significant alterations in acoustic properties of myocardium when compared with patients with EH. In their study, myocardial tissue characterization was performed by videodensitometric evaluation of standard echocardiographic images, and myocardial texture was evaluated as CV of the myocardial gray level amplitude. In the present study, acoustic intensity of the backscatter signal was analyzed, and both diastolic IBS and CV of IBS were used to describe myocardial texture and function. Compared with videodensitometric methods, analysis of IBS is considered more appropriate for evaluation of the acoustic properties of the myocardium, as it is done upstream the imaging chain and thus it is not influenced by the internal processing and interpolation algorithms used in standard echocardiographic imaging. Furthermore, CV of the ultrasonic signal reflects the changes in myocardial fiber orientation during the cardiac cycle and depends on the contractile performance of myocardium which can be altered both by changes in extracellular matrix and by enlargement of cardiac myocytes. Myocardial IBS level in diastole depends largely on an extracellular matrix, and correlates with myocardial collagen content, and therefore may represent a more direct estimate of myocardial fibrosis.

Discussion

The possible effect of aldosterone on myocardial performance is not obvious. Increased collagen content within myocardial interstitium can be presumed to impair contractile behavior of myocardial fibers; nevertheless, a direct inotropic effect of the excess of aldosterone has been proposed. Previous clinical studies have not confirmed the assumption that endogenously produced aldosterone exerts an inotropic effect on LV myocardium and demonstrated that in patients with EH, an inadequate suppression of aldosterone in response to salt loading was related to more significant impairment of midwall shortening. Our data suggest that aldosterone-induced alterations in myocardial composition and geometry result in a subtle impairment of myocardial performance. These alterations can also modify myocardial stiffness and therefore LV diastolic properties. In the present study, diastolic function was estimated indirectly through Doppler-derived indexes of LV filling. In agreement with previous data, patients with hyperaldosteronism had a greater dependence of LV filling from presystole, a finding that can be partially explained by a longer duration of PQ interval, possibly caused by hypokalemia. However, our observation that, with increasing myocardial IBS, acceleration of early LV diastolic filling decreases and the contribution of late, presystolic component increases, suggests a direct relation between impaired LV filling and myocardial texture alterations.
Study Limitations

The number of patients with secondary hypertension is relatively low because of the restrictive inclusion criteria used. We included only subjects with IBS images of good quality, even when obtained with machine settings kept at constant image depth and transmit power, without use of time and lateral gain compensations. Myocardial IBS acquired under these conditions can be used for between-subject comparisons. We excluded all patients previously treated by drugs known to modify myocardial fibrosis (spironolactone, ACE inhibitors, and angiotensin II antagonists).

A recent experimental study has demonstrated that in IBS images, signal attenuation caused by depth occurred with an average decrease in IBS of −2.34 ± 0.59 dB for each centimeter of the depth. In our population, the distance between ultrasonic probe and midportion of interventricular septum (where myocardial IBS was measured) was similar between groups, being 6.6 ± 0.6, 6.7 ± 0.7, 7.0 ± 0.8, and 7.1 ± 0.7 cm in normotensive control subjects and patients with EH, RVH, and PA, respectively. A slightly deeper location of interventricular septum in patients with RVH and PA should result in a slight, relative underestimation of myocardial IBS in patients with hyperaldosteronism and thus should not influence the conclusions of our study.

Conclusions

The present study demonstrates in humans an association between myocardial IBS and circulating aldosterone and immunoreactive ET. This association supports the hypothesis that aldosterone and ET induce alterations in myocardial texture, caused by the increase in extracellular matrix and collagen content. Alterations in myocardial texture are related to deterioration of myocardial systolic performance and unfavorable LV diastolic filling pattern.

Perspectives

Myocardial texture characterization performed by analysis of ultrasonic backscatter signal may contribute to the understanding of mechanisms involved in the development and progression of myocardial fibrosis and hypertrophy. In addition, this approach could be used to detect early changes in myocardial texture and function as well as to follow the effect of therapeutic interventions.

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References


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