Quantitative Ultrasonic Analysis of Myocardium in Patients With Thalassemia Major and Iron Overload

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Background. Patients with β-thalassemia major present with severe anemia and need continuous transfusion therapy. The consequent iron overload leads to hemochromatosis. Initial cardiac dysfunction has been documented even in thalassemics without clinical manifestations of heart failure as well as by conventional echocardiographic-Doppler techniques. The purpose of this study was to assess the acoustic quantitative properties of myocardium in patients with iron overload.

Methods and Results. Thirty-eight patients with β-thalassemia major, without clinical signs of cardiac failure, and 20 age- and sex-matched young controls were studied by echocardiography. An on-line analysis of the ultrasonic radiofrequency signal was performed to obtain quantitative operator-independent measurements of the integrated backscatter (IB) signal of the ventricular septum and the posterior wall. The integrated values of the radiofrequency signal were normalized for the pericardial interface and expressed in percent (IB%). Thalassemic patients had been receiving transfusion therapy for 16±5 years and had received 313±138 transfusion units; they all had received chelation treatment (desferrioxamine) for 9±2 years. Patients and controls showed comparable values of echocardiographically assessed percent fractional shortening (32±3% versus 36±4%, p=NS), whereas thalassemics showed higher values of left ventricular mass index (118±50 versus 98±15 g/m², p<0.05). The IB% values were higher in patients with thalassemia major than in controls for both septum (35±14% versus 21±6%, p<0.001) and posterior wall (16±6% versus 11±3%, p<0.001). In thalassemic patients, no significant correlation was found between the septum IB% value and hematological parameters, such as the total number of transfusions (r=0.2, p=NS) or the mean ferritin value (r=0.1, p=NS). No significant correlation was also found between the septum IB% value and the echocardiographically assessed left ventricular mass index (r=0.2, p=NS).

Conclusions. These data demonstrate that myocardial reflectivity is abnormally increased in patients with thalassemia major under transfusion treatment, probably due to myocardial iron deposits and/or secondary structural changes. These quantitatively assessed abnormalities in regional reflectivity can be detected when conventional echocardiographic parameters of systolic left ventricular function are undistinguishable from normal controls. (Circulation 1993;87:748–754)

KEY WORDS • echocardiography • tissue • thalassemia • hemochromatosis

Patients with β-thalassemia major present with severe anemia and need continuous transfusion therapy. The consequent iron overload, also caused by extravasal hemolysis and increased iron absorption, leads to hemochromatosis.1 Cardiac disorders related to biventricular failure are the most frequent cause of death in this syndrome.2 A few studies have documented initial cardiac dysfunction even in thalassemics without clinical manifestations of heart failure,3,4 also by means of conventional echocardiographic-Doppler techniques.5-7 However, the qualitatively assessed echocardiographic reflectivity of the myocardium remains unchanged in the early phase of the iron-overload disease and becomes clearly abnormal (with increased echogenicity of myocardial walls) only in a few thalassemic patients in the advanced stages of the disease (usually represented by dilated cardiomyopathy).8

Nonconventional quantitative ultrasound methods for tissue characterization have recently proved to be reliable in identifying progressive degrees of histopathological impairment of cardiovascular structures, also in humans.9,10 In particular, a significant correlation has been found in in vitro11-15 and in vivo models16,17 between radiofrequency backscattered ultrasound signal increase and the fibrotic and/or calcium content of the target. Moreover, abnormalities in both absolute and cyclic systolic-to-diastolic variations of the quantitatively evaluated radiofrequency reflections of myocardial walls are present in patients with cardiac diseases associated with histomorphological tissue abnormalities, such as cardiomyopathies.17-19

Table 1. Demographic Data of Patients With Thalassemia Major and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients with thalassemia major</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>No. of subjects</td>
<td>38</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>17.7±5.1</td>
<td>17.6±6.6</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>21/17</td>
<td>12/8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>47±14</td>
<td>57±12*</td>
</tr>
<tr>
<td>Body surface (m²)</td>
<td>1.40±0.27</td>
<td>1.61±0.31*</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>110±10</td>
<td>116±8</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>71±9</td>
<td>76±6</td>
</tr>
</tbody>
</table>

*p<0.05 vs. patients' data. Values are mean±SD.

The purpose of this study is to assess whether abnormal myocardial wall reflectivity is present in patients with β-thalassemia major who are receiving transfusion treatment but are free from symptoms due to cardiac dysfunction by means of simple ultrasound system for on-line quantitative evaluation of the radiofrequency backscattered signal.

Methods

Patient Population

Forty-two patients with β-thalassemia major (homozygous form) and no clinical signs of cardiac dysfunction were initially considered for the study. After conventional echocardiographic examination, four patients were excluded for instrumental signs of left ventricular dysfunction (end-diatostic diameter ≥56 mm and/or fractional shortening <30%). The remaining 38 patients were enrolled for conventional and quantitative ultrasound myocardial analysis. No patients were excluded because of a poor-quality acoustic window. Their mean age was 18 years (age range, 7–26 years); 21 of them were male (55%). Demographic data of patients are reported in Table 1.

Each patient was receiving blood transfusions every 2–3 weeks to maintain hemoglobin levels between 10.5 and 13.5 g/dL. They all were also receiving desferrioxamine as chelation therapy (25–50 mg/kg body wt s.c. infused 4–6 days a week to maintain serum ferritin level below 1,300 ng/mL) to prevent as well as to treat the iron toxicity.20 Hematological data of the patients at the time of ultrasound evaluation are summarized in Table 2.

Control Group

Twenty age- and sex-matched normal subjects were also studied. They had no personal and/or family history of cardiac or hematological disease and had normal clinical, ECG, and echocardiographic findings. Their mean age was 17 years (age range, 6–30 years); 12 of them were male (60%). Demographic data of patients are reported in Table 1.

Each patient and control underwent on the same day a conventional two-dimensional echocardiographic and transmirtal Doppler flow evaluation and a quantitative radiofrequency analysis.

Two-dimensional Echocardiography

M-mode and two-dimensional echocardiographic tracings were obtained in each subject using a Hewlett-Packard 77020AI phased-array sector scanner (Andover, Mass.) with 3.5- and 2.5-MHz transducers. Two-dimensional images were obtained in the parasternal long- and short-axis views and apical two- and four-chamber views. Left ventricular diameters and wall thicknesses were measured from the two-dimensional targeted M-mode echocardiographic tracings according to the criteria of the American Society of Echocardiography.21 Left ventricular percent fractional shortening was calculated as end-diastolic diameter minus end-systolic diameter divided by end-diastolic diameter and multiplied by 100. Left ventricular mass was calculated by the Penn convention method22 and normalized for body surface (left ventricular mass index).

The wall echoreflectivity was visually and qualitatively assessed by two independent observers as either normal or increased. In case of split decisions (which occurred in six cases), a third observer reviewed the study, and his judgment was binding.

The Doppler transmitral flow velocity profile was obtained from the apical four-chamber view. In each subject, four to six consecutive cardiac cycles with the highest diastolic flow velocity as well as the best signal-to-noise ratios were chosen for analysis. The ratio between the early and late peaks of flow velocity (E/A) was measured in each patient. This Doppler diastolic index has been shown to have satisfactory reproducibility and to be an early and consistent sign of left ventricular filling abnormalities in patients with thalassemia major.

Ultrasound Tissue Characterization

The quantitative analysis system was developed in our institution and has been used in a different study population.19 An ESAOTE Biomedica SIM 3000 two-dimensional mechanical sector scanner echocardiograph was used for spatial orientation of the ultrasound beam; quantitative analysis of ultrasonic reflectivity was performed in the regions of interest, i.e., the ventricular septum and the posterior wall of left ventricle. These regions were visualized in the parasternal long-axis view. The acquisition of the backscattered signal was performed at end diastole since a systematic variation in backscatter amplitude occurs during the cardiac cycle.23 A 3.5-MHz frequency transducer (focal distance, 7 cm; -3 dB focal region, 6 cm) was used.

The “native” radiofrequency signal was sampled before the processing chain of the two-dimensional instrument. The radiofrequency signal underwent preamplification, bypassing the receiving circuits of the ultrasonic equipment. The analog signal was fed to an amplifier, and the gain sweep of the amplifier (2–60 dB) was accomplished in 30 steps. This allows full use of the input dynamic range of the analog-to-digital converter. Sampling was performed by a flash converter with 8 bits
of amplitude resolution at a rate of 40 MHz. The digitized signal from analog-to-digital converter was analyzed in real time by a hardware prototype developed in our electronics laboratory. The two-dimensional acquisition gate was visualized on the two-dimensional image so as to ensure its proper positioning (Figure 1).

For analysis of the myocardium, the gate width was kept at 3 μsec, which corresponds to 2.35 mm (for 64 points), given the velocity of ultrasound in biological tissues of 1.57 mm/μsec. This allowed for sampling of the radiofrequency signal in the mid subendocardial layers of the myocardium, thus excluding epicardial and endocardial specular reflections. The acquisition gate was placed immediately behind the specular echo of the endocardium (left endocardium for the septum) to minimize the transmural variations in backscatter that are due to the position from which the signal is acquired within the wall. For evaluation of the pericardial echo, a 1.5-μsec gate length was used (which corresponds to 1.2 mm, for 32 points). The acquisition gate was centered on the strongest pericardial reflections, immediately behind the mitral leaflets. The representative value of reflectivity for both myocardial walls and pericardium was calculated as the mean of three measurements.

The hardware analysis involved the measurement of the integrated amplitude of the rectified radiofrequency signal corresponding to the two-dimensional area selected from the echocardiographic image.

More analytically, the two-dimensional integrated backscatter index (2D-IB) was calculated over a tissue area, i.e., corresponding to a (n-m) segment in depth and a (r-l) segment in lateral displacement, as follows:

$$2D\text{-}IB = \frac{1}{x_r-x_l} \sum_{j=r}^{l} IB(x_j)$$

where

$$IB(x_j) = \frac{1}{y_n-y_m} \sum_{i=m}^{n} |S(x_j, y_i)|$$

represents the processing over the depth of the interrogated tissue; $S(x_j, y_i)$ is the sequence of the digitized backscattered echoes over the selected two-dimensional area and is expressed in millivolts.

The system provides a simultaneous display of conventional information together with tissue characterization parameters (the 2D-IB alphanumeric index and the lateral displacement profile averaged over the selected depth). Alphanumeric 2D-IB data values are transferred on-line to a personal computer (model AT) for statistical analysis.

Ultrasonic Quantitative Data Analysis

Primary 2D-IB values are highly influenced by interpatient differences in chest morphology, heart structure depth, and ultrasonic impedance. Therefore, to quantitatively assess the reflectivity of the interventricular septum and posterior free wall, we used the percent 2D-IB (as related to pericardial interface).

In particular, 2D-IB results for each heart structure, initially displayed in millivolts, were expressed as percent values, assuming the pericardial interface (from which the peak echo intensity was consistently recorded in each patient) to be 100%. In this manner, the individual pericardial signal strength was used to normalize myocardial signals in each patient following this procedure: The primary 2D-IB measurement (in millivolts) of myocardial wall (septum or posterior wall) reflectivity is divided by the primary 2D-IB measurement (in millivolts) of pericardial reflectivity and the result is multiplied by 100 to obtain the percent integrated backscatter index.18,25,26
Table 3. Conventional Echocardiographic and Doppler Findings in Patients With Thalassemia Major and in Controls Subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients with thalassemia major</th>
<th>Controls</th>
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<tbody>
<tr>
<td>VS (mm)</td>
<td>9.3±1.7</td>
<td>8.7±1.7</td>
</tr>
<tr>
<td>PW (mm)</td>
<td>8.6±1.6</td>
<td>8.5±1.2</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>44.9±5.9</td>
<td>48.0±3.9</td>
</tr>
<tr>
<td>LVMi (g/m²)</td>
<td>118.4±29.9</td>
<td>98.4±15.1*</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>324±2.5</td>
<td>36.0±3.5</td>
</tr>
<tr>
<td>E wave (cm/sec)</td>
<td>93±16</td>
<td>86±9*</td>
</tr>
<tr>
<td>A wave (cm/sec)</td>
<td>42±11</td>
<td>44±8</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>2.4±0.7</td>
<td>2.0±0.5*</td>
</tr>
<tr>
<td>RR (msec)</td>
<td>797±124</td>
<td>824±105</td>
</tr>
</tbody>
</table>

*P<0.05 vs. patients’ data.

Together with the percent IB, the following three quantitative measurements were displayed: 1) the primary 2D-IB values (expressed in millivolts) for each structure; 2) the normalized 2D-IB values, expressed in dB (that is, a relative unit measurement of sound energy, conventionally used), of primary measurements referred to an external specular reflection (previously acquired, and with a very high reflectivity) (calculated as 20 log V/Vr, where V [value corresponding to the myocardial wall–septum or posterior wall] and Vr [value corresponding to the reference external reflection] are both primary 2D-IB values and expressed in millivolts); 3) the normalized 2D-IB values, expressed in dB, of primary measurements are referred in each patient to the pericardial reflection and calculated with the above-mentioned formula, replacing the external reflection with the internal pericardial interface for each subject.

Statistical Analysis

Data are given as mean±SD. Intergroup differences were tested for significance using the unpaired Student's t test. Relations between radiofrequency and two-dimensional echocardiographic measurements were expressed in terms of linear regression analysis. A value of P<0.05 was considered statistically significant.

Results

In all subjects, the conventional echocardiographic and radiofrequency quantitative ultrasound measurements were obtained.

Echocardiographic Findings

Echocardiographic and Doppler findings of patients and control subjects are shown in Table 3.

No abnormal values of left ventricular diameter, left ventricular mass index, or percent fractional shortening were found. No epicardial junction abnormalities were recorded.

No significant differences in two-dimensional-echocardiographic parameters were present between patients and control groups, although patients tended to have a higher left ventricular mass index—probably due to reduced body surface with respect to age-matched controls. The patients show a significantly higher, although normal, value of transmitral Doppler E/A ratio compared with controls, mainly due to an increased early diastolic flow velocity (E wave).

The qualitatively assessed echocardiographic reflectivity of myocardial walls was considered increased in 10 thalassemic patients (interventricular septum in three, posterior wall in one, and both in six) and in none of the controls (26% versus 0%, p<0.01). Pericardial interface echocardiographic appearance was qualitatively judged to be in the normal range in each study subject.

Quantitative Ultrasonic Backscatter Data

2D-IB primary, normalized, and percent values of myocardial wall in patients and controls are summarized in Table 4. To evaluate differences on analyzed heart structures depth in the chest wall, data on the distance from the ultrasound transducer to the acquisition gate on septum, posterior wall, and pericardium, in both patients and control groups, are also presented in Table 4. The analysis of these data demonstrates that heart structures of patients and controls have no significant differences in the depth value.

Percent backscatter values of both ventricular septum and posterior wall in patients with thalassemia major showed highly significant differences compared with control subjects, exhibiting a diffuse increase in quantitatively assessed myocardial reflectivity (Figure 2).

In both patients and controls, the ventricular septum showed higher reflectivity than the posterior wall. This
finding is consistent with the result obtained in other studies where the same analysis system was used and is very likely due to the different distance of the insonated structures from the focal point of the ultrasonic beam as well as to the different attenuation of the signal.

**Relation Between Conventional Echocardiographic and Quantitative Backscatter Results**

In patients with thalassemia major, the relation between percent 2D-IB values of the myocardial walls and conventional echocardiographic measurements was analyzed. No significant correlation was found between quantitative reflectivity and thickness of ventricular septum and posterior wall \( (r=0.1 \text{ and } 0.1, \text{ respectively;} \ p=\text{NS for both}) \). No significant correlation was found when comparing septal and posterior wall percent 2D-IB and left ventricular end-diastolic diameter \( (r=0.1 \text{ and } 0.2, \ p=\text{NS}) \) or left ventricular mass index \( (r=0.2 \text{ and } 0.2, \ p=\text{NS}) \) or percentual fractional shortening \( (r=0.2 \text{ and } 0.1, \ p=\text{NS}) \) or E/A ratio \( (r=0.3 \text{ and } 0.1, \ p=\text{NS}) \).

The 10 thalassemic patients with qualitatively assessed increased echocardiographic appearance of septum and posterior wall did not exhibit higher values of parietal 2D-IB compared with thalassemic patients with normally appearing reflectivity (for septum: \( n=9, 42.0\pm16.3\% \text{ versus } 32.6\pm12.2\%, \ p=\text{NS} \); for posterior wall: \( n=7, 17.0\pm6.5\% \text{ versus } 16.1\pm5.3\%, \ p=\text{NS} \)).

**Relation Between Hematological Profile and Quantitative Backscatter Results**

Hematological data of patients with \( \beta \)-thalassemia major were compared with septal and posterior wall percent 2D-IB values for correlation. No significant correlation was identified when considering the years of transfusion therapy \( (r=0.1 \text{ compared with septal percent } 2\text{D-IB and } 0.04 \text{ compared with posterior wall percent } 2\text{D-IB, } p=\text{NS for both}) \), the total number of transfusions \( (r=0.2 \text{ and } 0.1, \text{ respectively; } p=\text{NS}) \), the years of chelation therapy \( (r=0.1 \text{ and } 0.1, p=\text{NS}) \), or the mean ferritin value \( (r=0.1 \text{ and } 0.1, p=\text{NS}) \) (Figure 3).

**Discussion**

The results of this study clearly demonstrate that in patients with \( \beta \)-thalassemia major, even without clinical signs of cardiac functional involvement, there are ab-normalities in myocardial acoustic properties, probably related to histopathological alterations. The increased reactivity of myocardial walls found in these patients might be technically explained by several morphological substrates that have been documented by histological and biochemical studies in cardiac hemochromatosis: fibrosis, hypertrophy, and iron deposition.1–3 Histopathological changes of heart structures due to secondary iron overload are characterized mainly by the presence of small iron deposits, predominantly within cells of ventricular myocardium and conduction tissue.
deposits are more diffuse in the subepicardial than in the subendocardial regions; fogosis and fibrosis are minimal to mild. Macroscopic alterations consist of left ventricular hypertrophy and, in the late stages of the disease, dilation of heart chambers. Pericardial effusion can be observed in the advanced stages.

The collagen content is a major determinant of myocardial backscatter, as shown by theoretical evidence as well as by experimental and clinical studies. In humans, a significant relation has been demonstrated between the amount of connective tissue—morphometrically evaluated from endomyocardial biopsies—and the intensity of regional myocardial backscatter. In hemochromatosis, connective tissue may be increased, which may account for the observed increase in myocardial ultrasonic reflectivity. However, in this pathology, the entity of myocardial fibrosis is usually mild, and therefore other causes are likely to contribute to increased echo density.

Left ventricular hypertrophy might be another cause of abnormal myocardial acoustic characteristics. In previous studies, we have found an increased echoreflectivity in patients with hypertrophic cardiomyopathy but not in the physiological myocardial hypertrophy of athletes, suggesting that increased echoreflectivity in hypertrophic cardiomyopathy is probably related to fiber disarray as well as to fibrosis and necrosis foci. The myocardial hypertrophy at times present in hemochromatosis is not associated with fiber disarray; therefore, such morphological changes are not likely to be a major determinant of regional echodensity—partly because left ventricular wall thickness was normal in our study population.

Finally, intracellular iron deposition in hemochromatosis might be responsible for the increased reflectivity of myocardial walls since multiple foci of intracellular iron deposition might create discontinuities in acoustic impedance and generate multiple interfaces, eventually increasing the ultrasonic backscatter. Iron deposition most likely occurs in the heart of patients undergoing frequent blood transfusions, such as thalassemics. In the patients of the present study, however, no significant correlation was found between acoustic quantitative parameter (spectral backscatter) and hematological data (years and number of blood transfusions, years of chelation therapy, and mean serum ferritin value). This finding, although seemingly surprising, is in accordance with reports by others who did not find a correlation between hematological parameters and myocardial involvement. The mechanism by which iron overload produces tissue damage has not been well established, but it is generally accepted that iron toxicity begins when the iron load exceeds the tissue- or blood-binding capacity and joins a free pool defined as nontransferritin iron. The amount of iron that can be stored in a tissue depends on the capacity of the tissue to generate storage proteins; as we are not able to evaluate the amount of free pool, only a rough correlation can be derived between storage levels and cardiac toxicity. Therefore, myocardial involvement may occur in the absence of excessive cardiac stores, partly because heart cells have a relatively small amount of storage proteins and are very sensitive to free iron–induced oxygen radicals that are directly responsible for cellular damage.

Furthermore, it is likely that the system used for quantitative ultrasound analysis can detect the increase in echoreflectivity due to abnormal iron deposition in the myocardium but is not able to identify little variations in the entity of these deposits. Moreover, the entity and progression of fibrotic reaction, another important cause of increased echoreflectivity, can be independent (not correlated) to the entity of iron deposition, which may represent the initial trigger of that reaction.

Thus, the increased echoreflectivity detected in the myocardium of thalassemia major patients might be multifactorial in origin and related to fibrosis, iron stores, or both. The relative role of each factor is difficult to establish in our patients, particularly because no histopathological correlation was available.

The only reliable method to obtain such data in a clinical study, such as this one, is by the use of endomyocardial biopsy, whose results would have allowed a direct correlative analysis between ultrasonic and histological findings. However, this invasive procedure carries a definite risk and is not routinely performed in patients with thalassemia major since no significant beneficial effect on the clinical management of these patients can be obtained through the analysis of its results.

Although echocardiographic indexes of regional and global systolic function were normal in our thalassemic patients, an increased E/A ratio versus normals was detected by Doppler echocardiography. This finding is consistent with previous observations by Spirito et al., who showed restrictive diastolic abnormalities by transmitial Doppler in these patients—consisting of increased E wave and reduced flow–velocity deceleration time—in an early phase of cardiac involvement, when symptoms of heart failure are absent and systolic function is normal.

Of interest, the qualitatively assessed reflectivity on two-dimensional echocardiograms did not clearly differentiate patients with markedly increased regional backscatter. This is, however, not surprising in view of the basically different nature of the information provided by radiofrequency backscatter compared with the image brightness of standard two-dimensional images. In fact, the electronic processing in commercially available two-dimensional imaging systems heavily manipulates the radiofrequency native signal to optimize the display of specular reflectors, such as endocardial borders. The processes of differentiation, thresholding, nonlinear amplification, and overall and regional gain are aimed at generating a clearly defined, pleasant image but certainly disrupt the linear relation between the signal received by the transducer and the signal displayed in the echo monitor. A sampling of the backscattered myocardial signal, although much more complex, allows a more accurate characterization of myocardial acoustic properties.

In conclusion, although further studies are needed to confirm our observation, we emphasize that myocardial tissue characterization with a real-time integrated backscatter imaging system is a useful tool for detecting myocardial abnormalities in patients with thalassemia major before clinical signs of cardiac damage and echocardiographic evidence of left ventricular regional and global dysfunction are detectable.
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