On T2* Magnetic Resonance and Cardiac Iron

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Background—Measurement of myocardial iron is key to the clinical management of patients at risk of siderotic cardiomyopathy. The cardiovascular magnetic resonance relaxation parameter T2* (assessed clinically via its reciprocal, T2) measured in the ventricular septum is used to assess cardiac iron, but iron calibration and distribution data in humans are limited.

Methods and Results—Twelve human hearts were studied from transfusion-dependent patients after either death (heart failure, n=7; stroke, n=1) or transplantation for end-stage heart failure (n=4). After cardiovascular magnetic resonance T2* measurement, tissue iron concentration was measured in multiple samples of each heart with inductively coupled plasma atomic emission spectroscopy. Iron distribution throughout the heart showed no systematic variation between segments, but epicardial iron concentration was higher than in the endocardium. The mean±SD global myocardial iron causing severe heart failure in 10 patients was 5.98±2.42 mg/g dry weight (range, 3.19 to 9.50 mg/g), but in 1 outlier case of heart failure was 25.9 mg/g dry weight. Myocardial ln[R2*] was strongly linearly correlated with ln[Fe] (R²=0.91, P<0.001), leading to [Fe]=45.0×(T2*)⁻¹. The clinical calibration equation with [Fe] in milligrams per gram dry weight and T2* in milliseconds. Midventricular septal iron concentration and T2* were both highly representative of mean global myocardial iron.

Conclusions—These data detail the iron distribution throughout the heart in iron overload and provide calibration in humans for cardiovascular magnetic resonance T2 against myocardial iron concentration. The iron values are of considerable interest in terms of the level of cardiac iron associated with iron-related death and indicate that the heart is more sensitive to iron loading than the liver. The results also validate the current clinical practice of monitoring cardiac iron in vivo by cardiovascular magnetic resonance of the midseptum. (Circulation. 2011;123:1519-1528.)

Key Words: heart • iron overload • magnetic resonance imaging • siderosis • thalassemia

Transfusion-dependent patients receive ~20 times the normal intake of iron, which leads to iron accumulation and damage in the liver, heart, and endocrine organs. Although improvements in survival have been achieved over the last 40 years, iron-induced cardiotoxicity remains a leading cause of morbidity and mortality, especially for patients with β-thalassemia major, and once heart failure develops, the prognosis is poor. Therefore, assessment of myocardial iron is essential clinically, but conventional non-invasive techniques are less than ideal. Neither serum ferritin nor liver iron concentration gives a reliable indication of cardiac iron in cross-sectional studies. Monitoring ejection fraction can be useful, but its value is limited by the difficulty of obtaining reproducible longitudinal measurements, the masking of ventricular dysfunction by the basal high cardiac output seen in chronic anemia, and its late occurrence in the disease process. Even more modern contractile measures, such as tissue Doppler imaging, correlate poorly with cardiac iron. Endomyocardial biopsy is also unreliable for measuring myocardial iron because of sampling error with very small biopsy samples. The need for an alternative noninvasive measurement of myocardial iron led to the development of an optimized cardiac T2 magnetic resonance (MR) technique. Particular intracellular iron causes shortening of the MR...
relaxation parameter $T2^*$ (and hence an increase in its reciprocal, $R2^*$) owing to microscopic magnetic field inhomogeneity. Myocardial $T2^*$ is an easily quantifiable, clinically robust, and highly reproducible measurement technique. In the liver, $T2^*$ correlates well with biopsy iron concentration. Although the relationship between $T2^*$ and cardiac iron can be inferred from first principles, animal models, and the liver data, there is limited direct information on cardiac calibration of $T2^*$ in humans, and the inherent limitations associated with endomyocardial biopsy prevent in vivo calibration. We therefore initiated a project to calibrate cardiac $T2^*$ against chemically assayed tissue iron concentration in ex vivo human hearts and to determine its cardiac distribution.

Clinical Perspective on p 1528

Methods

Patient Characteristics

This project was started in 2003 and completed in 2010, during which time 12 whole human hearts were donated from 5 international centers: University College Hospital (London, United Kingdom), Ospedale Galleria (Genoa, Italy), Children’s Hospital of Los Angeles (United States), Azienda Ospedaliera Brotzu (Cagliari, Italy), and Mahidol University (Bangkok, Thailand). All patients were transfusion dependent (10 with $\alpha$-thalassemia major, 1 with sideroblastic anemia, and 1 with Diamond Blackfan anemia), most having required transfusions from early childhood. The hearts were donated after death or cardiac transplantation for end-stage heart failure. The study protocol was approved by all local research ethics committees, and local consent was obtained in all cases.

Imaging Protocol and Analysis

We used scientific convention in quoting $R2^*$ to evaluate relaxation and its relation to iron, $R2^*$ being the reciprocal of $T2^*$. Scans performed on patients premortem are quoted as $T2^*$. All hearts were fixed in formalin. Each heart was cut into 4 to 5 slices of 1-cm thickness in the ventricular short axis according to ventricular size. Although the relationship between $T2^*$ and cardiac iron can be inferred from first principles, animal models, and the liver data, there is limited direct information on cardiac calibration of $T2^*$ in humans, and the inherent limitations associated with endomyocardial biopsy prevent in vivo calibration. We therefore initiated a project to calibrate cardiac $T2^*$ against chemically assayed tissue iron concentration in ex vivo human hearts and to determine its cardiac distribution.

Figure 1. Experimental setup. A, Diagram of short-axis cardiac slices showing regions of interest (ROIs). B, Set of slices from a single heart. C, Slices inside scanner. D, Ex vivo cardiac magnetic resonance image with ROIs used to determine $T2^*$ decay. E, Identical slice with segmentation performed to correspond with ROIs used for $T2^*$ measurement.

Figure 2. Example of ex vivo cardiac $T2^*$ scans. Representative images of 2 hearts are shown at increasing echo times (TE) from 3 to 17.4 milliseconds. Heart 1 has normal iron levels and remains bright, whereas heart 2 (which has severe iron loading) shows progressive darkening with increasing echo time. The graph shows signal intensity (arbitrary units) plotted against echo time (milliseconds) for the hearts shown on top. Heart 1 has a shallow decay curve with $T2^*$ value of 20 milliseconds. Heart 2 has a much more rapid decay with $T2^* < 10$ milliseconds.
copy. The iron concentrations in samples of NIST human liver
the lyophilizer. After acid digestion, iron measurement was per-
the dry weight (dw) was recorded immediately after removal from
excess formalin was discarded. Samples were then freeze-dried, and
analyzed together and the 2 apical-lateral sectors were analyzed
together. The wet weight of each piece of tissue was recorded after

extent of myocardium. To match the apical slice to 4 segments as
slices, as per the model. Each segment comprised the full transmural
were used for this analysis: the midventricular, apical, and basal

DBA, Diamond Blackfan anemia; and N/A, not applicable (patients 3 and 4 did not receive iron chelation therapy).

For the measurement of R2*, each slice was analyzed with 18 regions of interest (ROIs) of 6 radial sectors of 60° each and 3 layers: outer (epicardial), inner (endocardial), and intermediate (mesocar-
dial; Figure 1D). The attachment of the right ventricular (RV) wall to
the left ventricle (LV) was used to define the septum.20 R2* was
measured from each ROI with dedicated software (Thalassaemia
Tools, Cardiovascular Imaging Solutions, London, UK) using a
truncation model to account for background noise, as has been
validated previously (Figure 2).21 Analysis of the 12 echo sequence
was required only for heart 4, which was extremely heavily iron
loaded (R2*, 500 seconds⁻¹; T2* 2.0 milliseconds). In all other
cases, the 16-echo sequence was used for analysis.

Quantification of Myocardial Iron

After R2* cardiac MR was completed, each short-axis slice was cut
into 6 sectors of 60° each, and then each sector was subdivided to
3 transmural layers (18 LV samples; Figure 1E). For each short-axis
slice, 2 samples were also taken from the RV free wall (20
myocardial samples per slice). Additional samples were taken from
the right (3) and left (3) atria, the interatrial septum (1), and each of
the valves (4). For the LV myocardial R2* calibration analysis, all
LV samples were directly compared with the cardiac MR R2* scan.
For the segmental analysis of the distribution of myocardial iron, we
used the American Heart Association/American College of Cardiol-
ogy 16-segment model.20 Three myocardial slices from each heart
were used for this analysis: the midventricular, apical, and basal
slices, as per the model. Each segment comprised the full transmural
extent of myocardium. To match the apical slice to 4 segments as
dicted in the 16-segment model, the 2 apical-septal sectors were
analyzed together and the 2 apical-lateral sectors were analyzed
together. The wet weight of each piece of tissue was recorded after
excess formalin was discarded. Samples were then freeze-dried, and
the dry weight (dw) was recorded immediately after removal from
the lyophilizer. After acid digestion, iron measurement was per-
formed with inductively coupled plasma atomic emission spectro-
scopy, The iron concentrations in samples of NIST human liver
standard 4352 were used as quality controls for inductively coupled
plasma atomic emission spectroscopy analysis.

Effect of Time in Formalin Fixation on R2*
Measurement and Iron Concentration

Because the fixed hearts had been stored for various lengths of time,
the effect of formalin on MR relaxation and iron loss over time was
studied. One myocardial slice was analyzed at repeated intervals up
to a total of 566 days (12 scans in total). Myocardial tissue from an
apical slice not included in the segmental analysis was used to assess
whether there was any leaching of iron from the myocardium into
formalin over time. This 7.35-g sample was preserved in 280 mL of
10% neutral buffered formalin (pH 7.0). At repeated intervals up to
600 days, small (1 mL) aliquots of formalin were taken for analysis
of the iron content of the solution. The initial iron concentration in
this tissue sample was estimated from immediately adjacent areas of
myocardium in the same apical slice.

Premortem and Postmortem Scans

Three of the patients had clinical T2* scans shortly before death on
the same scanner (Sonata) used for the ex vivo scanning. Imaging
was performed with a single-breath-hold 8-echo T2* sequence, as
previously described (range of echo times, 2.6 to 16.74 milliseconds;
slice thickness, 10 mm; flip angle, 20°; matrix, 128×256 pixels; field
of view, 400 mm; and sampling bandwidth, 815 Hz per pixel).13 The
anatomic appearance of the slice (papillary muscle position, trabec-
ular pattern, and distance from apex) was used to achieve as precise
a correlation as possible between premortem and postmortem scans,
allowing direct comparison of T2* values. Two midventricular
short-axis slices were imaged, which gave 12 myocardial ROIs for
comparison (2 slices, 2 septal sectors, and 3 transmural layers).

Statistics

For each distribution analysis, 1 ROI was defined as the reference
region, and differences in tissue iron content throughout the heart
against this region were assessed with mixed-model linear regres-
son. The null hypothesis was that there was no difference in iron
concentration between segments, layers, or slices. For each analysis,
the ROIs were entered as fixed effects so that differences between
regions could be estimated. Patients were entered as random effects
because testing for differences between individual patients was not
of interest. To account for the repeated measurements from each
heart, all samples from each individual heart were nested for
analysis. R2* was compared with iron concentration by linear
regression. The degree of heterogeneity of iron concentration and
R2* for each heart was evaluated by calculating the coefficient of
variation of iron concentration across all samples. All data were
analyzed with STATA version 10.1 (Stata Corp, College Station,
TX). Mixed-model linear regression analysis was performed with the
“xtmixed” command within STATA. The “regress” command was
used for standard linear regression. A value of P<0.05 was used to
define a significant difference.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Death or Cardiac Transplantation Age at Death or Transplantation, y</th>
<th>Cause of Death or Indication for Transplantation</th>
<th>LV [Fe] (Mean±SD), mg/g dw</th>
<th>Variability of [Fe], CoV %</th>
<th>Global Myocardial T2* (Mean±SD), ms</th>
<th>Total Estimated Units Transfused</th>
<th>Age Started Chelation, y</th>
<th>Compliance</th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>TM</td>
<td>Death 46</td>
<td>Ischemic stroke (no cardiac failure)</td>
<td>0.38±0.13</td>
<td>21.1</td>
<td>44.4±5.3</td>
<td>1584</td>
<td>6</td>
<td>Excellent</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>SA</td>
<td>Death 62</td>
<td>Cardiac failure</td>
<td>8.20±1.44</td>
<td>15.9</td>
<td>4.7±0.6</td>
<td>2000</td>
<td>42</td>
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</tr>
<tr>
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<td>M</td>
<td>TM</td>
<td>Death 10</td>
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<td>9.50±1.88</td>
<td>9.2</td>
<td>3.7±0.6</td>
<td>170</td>
<td>N/A†</td>
<td>N/A</td>
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<tr>
<td>4</td>
<td>F</td>
<td>TM</td>
<td>Death 15</td>
<td>Cardiac failure</td>
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<td>17.6</td>
<td>2.0±0.4</td>
<td>288</td>
<td>N/A†</td>
<td>N/A</td>
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<tr>
<td>5</td>
<td>F</td>
<td>TM</td>
<td>Death 20</td>
<td>Cardiac failure</td>
<td>7.7±1.51</td>
<td>16.4</td>
<td>3.6±0.5</td>
<td>432</td>
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<td>Poor</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>TM</td>
<td>Transplant 23</td>
<td>Cardiac failure</td>
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<td>18.0</td>
<td>8.0±1.0</td>
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<td>Not known</td>
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<tr>
<td>7</td>
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<td>TM</td>
<td>Transplant 24</td>
<td>Cardiac failure</td>
<td>3.38±0.53</td>
<td>10.0</td>
<td>7.7±1.2</td>
<td>528</td>
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<td>Good</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>TM</td>
<td>Transplant 21</td>
<td>Cardiac failure</td>
<td>5.87±1.0</td>
<td>9.5</td>
<td>4.4±0.6</td>
<td>500</td>
<td>4</td>
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</tr>
<tr>
<td>9</td>
<td>F</td>
<td>TM</td>
<td>Transplant 31</td>
<td>Cardiac failure</td>
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<td>10.5</td>
<td>3.9±0.6</td>
<td>624</td>
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</tr>
<tr>
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<td>M</td>
<td>TM</td>
<td>Transplant 24</td>
<td>Cardiac failure</td>
<td>5.64±1.36</td>
<td>17.3</td>
<td>5.8±1.4</td>
<td>660</td>
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<tr>
<td>11</td>
<td>M</td>
<td>TM</td>
<td>Death 44</td>
<td>Cardiac failure</td>
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<td>26.6</td>
<td>12.3±2.6</td>
<td>1209</td>
<td>18</td>
<td>Poor</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>DBA</td>
<td>Death 22</td>
<td>Cardiac failure and pneumonia</td>
<td>3.91±0.86</td>
<td>10.1</td>
<td>8.8±1.8</td>
<td>442</td>
<td>10</td>
<td>Poor</td>
</tr>
</tbody>
</table>

LV indicates left ventricle; dw, dry weight; [Fe], myocardial iron concentration; CoV, coefficient of variation; TM, β-thalassemia major; SA, sideroblastic anemia; DBA, Diamond Blackfan anemia; and N/A, not applicable (patients 3 and 4 did not receive iron chelation therapy).

*Died in 1964.
†Died in 1972.
Results

Patient Characteristics
Twelve hearts were donated, 8 from patients who died and 4 from patients undergoing cardiac transplantation for end-stage heart failure. End-stage heart failure was the cause of death in 7 of the 8 patients who died, but 1 patient died of an ischemic stroke at 46 years of age with a history of excellent compliance to chelation therapy and no cardiac complications (apart from a transient episode of atrial fibrillation at 21 years of age). Table 1 summarizes the clinical information for each patient.

Premortem and Postmortem Scans
Three patients underwent both premortem and postmortem T2* scans. The mean ± SD time between cardiac MR scan and death was 26 ± 13.3 days (range, 11 to 35). There was no significant difference in T2* between scans. T2* premortem was 4.89 ± 1.30, 6.01 ± 0.81, and 10.87 ± 2.34 milliseconds versus 5.21 ± 0.43 milliseconds (P = 0.28), 5.94 ± 0.90 milliseconds (P = 0.84), and 9.20 ± 2.22 milliseconds (P = 0.21) postmortem for pairwise comparison of the septal ROIs in hearts 2, 10, and 11, respectively (Figure 3A through 3C).

Effect of Time in Formalin on R2* Measurement
For the single short-axis slice that was scanned at repeated intervals, there was no significant change in R2* value, even after 18 months’ storage in formalin (Figure 3D). Mean R2* for all sectors was 198.4 ± 21.5 seconds⁻¹ at day 6 and 197.7 ± 7.3 seconds⁻¹ at day 566 (P = 0.93).

Loss of Iron From Stored Myocardial Samples
The iron content of the formalin solution in which a piece of myocardial tissue had been stored over a period of 600 days is shown in Figure 3E. The average iron concentration of adjacent myocardial tissue was 1.21 mg/g wet weight, corresponding to a total of 8.91 mg iron in the 7.35-g wet weight sample. The iron concentration of the solution increased rapidly over the first 30 days after formalin fixation with a further subsequent rise, but thereafter stabilized to reach a plateau of 480 g/L after 300 days, equating to a loss of 135 g (~1.5%) of the total iron from the myocardial tissue.

Myocardial Iron Samples: Overall Summary
The mean ± SD weight of all samples in the iron analysis was 1001 ± 620 mg. Iron concentration in all samples ranged from 0.09 to 78.64 mg/g dw. The myocardial iron concentration ranged from 0.18 to 53.4 mg/g dw. The mean ± SD ratio of dry to wet iron concentration in the myocardium was 5.04 ± 0.9 and was independent of iron concentration. One heart had exceptionally high levels of iron, and in view of the possibility that this heart could bias the results, all further analysis is presented separately for this outlier heart. The coefficient of variation of iron for myocardial samples ranged from 9.2% to 26.6% per heart with a mean of 15.2% (Table 1).
Distribution of LV Iron
With all hearts considered together (except for heart 4, the most heavily iron-loaded heart), a gradient of iron was found in the transmural layers of the LV myocardium with the greatest concentration in the epicardium, an intermediate level in the mesocardium (−1.1%; \( P=0.63 \)), and the lowest level in the endocardium (−7.5%; \( P=0.001 \); Figure 4A and Table 2). No statistically significant systematic variations in iron concentration were observed between the different AHA segments. To examine for variation in larger regions of the LV, an analysis was also performed of variation in iron concentration of whole walls of the LV (inferoseptal, anteroseptal, anterior, anterolateral, inferolateral, and inferior) encompassing all segments from base to apex, which again showed no significant systematic variation in mean iron concentration. A further analysis comparing the whole basal, midventricular, and apical slices also failed to show any significant systematic variation in mean iron concentration. The most heavily iron-loaded heart (heart 4) had very high iron concentration in the epicardium (36.1 mg/g dw), with an intermediate level in the endocardium (−28.0%) and the lowest level in the mesocardium (−54.7%; Figure 4A and Table 2). There was no systematic variation in mean iron concentration between the different LV segments or from base to apex.

Septal Iron Compared With Global Cardiac Iron
Iron concentration measured in the midseptal slice was highly representative of global iron (Figure 4B). Regression analysis showed a slope indistinguishable from 1 (1.06; 95% confidence interval [CI], 0.83 to 1.29) and an intercept indistinguishable from 0 (0.04; 95% CI, −1.57 to 1.66). The mean±SD percentage difference between midseptal and global iron concentration was 10.5±13.4%.

Distribution of Iron in the RV, Atria, and Valves
Excluding the most heavily iron-loaded heart (heart 4), all RV, atrial, and valve samples showed lower iron levels compared with global LV iron (apart from the anterior left atrial wall where only a borderline difference was observed). The RV wall had up to 21.9% less iron (\( P<0.001 \)). Compared with the mean global LV iron concentration, the left atrial iron concentration was up to 28.1% lower (\( P<0.001 \)) and right atrial iron was up to 57.0% lower (\( P<0.001 \)). All 4 cardiac valves showed low iron concentrations that were up to 74.4% lower than in the LV (\( P<0.001 \)). Heart 4 showed a consistently higher level of iron in the atrial and valve samples but no difference between LV and RV iron (Table 3).

Relation of \( R_2^* \) to Tissue Iron Concentration
Because of the very high level of iron in the most severely overloaded heart (heart 4), measurement of \( R_2^* \) might be underestimated. Therefore, the results of the MR relaxation calibration are presented twice, first including and then excluding this heart. A total of 1006 tissue samples and their corresponding ROIs were used for iron and \( R_2^* \) assessment, with 84 ROIs (8.3%) being excluded from the \( R_2^* \) analysis because of imaging artifact caused predominantly by cuts made at autopsy. A curvilinear relation between \( R_2^* \) and cardiac iron concentration was observed (Figure 5A and 5B). A similar curvilinear relation was shown when mean global whole-heart iron was plotted against \( R_2^* \) (Figure 5C and 5D). The strongest linear correlation was found (all hearts) by plotting \( \ln R_2^* \) and \( \ln [Fe] \) (\( R^2=0.910, P<0.001 \)) with a slope of 0.745 (95% CI, 0.740 to 0.750) and intercept of 3.896 (95% CI, 3.868 to 3.924; Figure 5E). There was almost no difference in this calibration result when heart 4 was excluded (\( R^2=0.898, P<0.001 \); slope, 0.754 [95% CI, 0.737 to 0.772]; intercept, 3.884 [95% CI, 3.855 to 3.913]). Using this calibration in all hearts, we can express the relation between \( R_2^* \) and cardiac iron concentration (Figure 5A and 5B)

\[ R_2^* = 0.00985 \times \text{[Fe]}^{-1.22} \]

\( R_2^* \) measured in the midseptal slice was highly representative of whole-heart mean \( R_2^* \) (Figure 5F). Regression analysis showed a slope indistinguishable from 1 (0.97; 95% CI, 0.82 to 1.13) and an intercept indistinguishable from 0 (0.33; 95% CI, −0.73 to 1.38). The mean±SD percentage difference between midseptal and global \( R_2^* \) was 2.8±7.3%. The relation of septal \( R_2^* \) and \( T_2^* \) with whole-heart iron concentration is shown in Figure 6.
Discussion

This is the first report of a series of human hearts that have undergone both MR relaxometry and quantitative iron studies for distribution and calibration purposes. The results of the distribution analysis show no systematic variation of iron concentration between all 16 myocardial segments, the 6 myocardial walls, and the 3 short-axis slices from base to apex. However, systematically higher iron concentration was present in the midventricular septal iron concentration was highly representative of global iron concentration, and midventricular septal R2* was highly representative of global R2*.

Studies dating from the mid-1950s have assessed myocardial iron loading in postmortem human hearts using various techniques, including histological grading of iron-positive myocytes, electron microscopy, and analysis of elemental iron by flameless atomic absorption spectroscopy.11,22–27 Our results are in broad agreement with the previously published studies, which have observed a similar transmural gradient of myocardial iron. Two autopsy studies have attempted to quantify regional variation, but no definite pattern has emerged,17,22 and this is the first comprehensive study of regional variation.

Cardiac MR studies have reported that some β-thalassemia major patients have a heterogeneous pattern of cardiac iron distribution as measured by in vivo T2*, although this heterogeneity lessens with increased iron concentration.28-29 Myocardial T2* in vivo, however, may be confounded by a number of factors, including magnetic susceptibility artifact and measurement error.30 In view of the problems with artifact in vivo, it has been recommended that the measurement of T2* be restricted to a full-thickness septal ROI.3 The results of the calibration analysis of myocardial MR relaxometry against absolute iron concentration are of considerable clinical interest. Heart R2* provided a robust ($R^2=0.910$) calibration against chemically assayed cardiac iron concentration.31,32

Table 2. Distribution of Iron Throughout the Left Ventricular Myocardium

<table>
<thead>
<tr>
<th>LV Region of interest</th>
<th>All Hearts (Excluding Heart 4)</th>
<th>Heart 4 Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Fe], mg/g</td>
<td>Lower 95% CI</td>
</tr>
<tr>
<td>Epicardium (reference)</td>
<td>6.17</td>
<td>4.65</td>
</tr>
<tr>
<td>Mesocardium (Δ)</td>
<td>-0.07</td>
<td>-0.34</td>
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<tr>
<td>Endocardium (Δ)</td>
<td>-0.46</td>
<td>-0.73</td>
</tr>
<tr>
<td>Global LV (reference)</td>
<td>6.01</td>
<td>4.30</td>
</tr>
<tr>
<td>Basal</td>
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</tr>
<tr>
<td>1 Ant (Δ)</td>
<td>-0.37</td>
<td>-1.30</td>
</tr>
<tr>
<td>2 Ant-sep (Δ)</td>
<td>0.09</td>
<td>-0.84</td>
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<tr>
<td>3 Inf-sep (Δ)</td>
<td>-0.27</td>
<td>-1.20</td>
</tr>
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<td>4 Inf (Δ)</td>
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<td>-1.34</td>
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<td>5 Inf-lat (Δ)</td>
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<td>-1.07</td>
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<tr>
<td>6 Ant-lat (Δ)</td>
<td>0.38</td>
<td>-0.55</td>
</tr>
<tr>
<td>Mid</td>
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<td></td>
</tr>
<tr>
<td>7 Ant (Δ)</td>
<td>0.03</td>
<td>-0.90</td>
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<td>8 Ant-sep (Δ)</td>
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<tr>
<td>14 Sep (Δ)</td>
<td>-0.29</td>
<td>-1.22</td>
</tr>
<tr>
<td>15 Inf (Δ)</td>
<td>-0.12</td>
<td>-1.05</td>
</tr>
<tr>
<td>16 Lat (Δ)</td>
<td>-0.50</td>
<td>-1.43</td>
</tr>
<tr>
<td>Global LV (reference)</td>
<td>5.99</td>
<td>4.28</td>
</tr>
<tr>
<td>Anterior (Δ)</td>
<td>-0.07</td>
<td>-0.93</td>
</tr>
<tr>
<td>Anterolateral (Δ)</td>
<td>0.16</td>
<td>-0.70</td>
</tr>
<tr>
<td>Inferolateral (Δ)</td>
<td>-0.06</td>
<td>-0.91</td>
</tr>
<tr>
<td>Inferior (Δ)</td>
<td>-0.16</td>
<td>-1.02</td>
</tr>
<tr>
<td>Infersesval (Δ)</td>
<td>-0.06</td>
<td>-0.92</td>
</tr>
<tr>
<td>Anterolesval (Δ)</td>
<td>0.20</td>
<td>-0.66</td>
</tr>
<tr>
<td>Apical (Δ)</td>
<td>0.27</td>
<td>-0.55</td>
</tr>
<tr>
<td>Mid (Δ)</td>
<td>-0.17</td>
<td>-1.00</td>
</tr>
<tr>
<td>Basal (Δ)</td>
<td>-0.11</td>
<td>-0.93</td>
</tr>
</tbody>
</table>

LV indicates left ventricular; CI, confidence interval; and [Fe], myocardial iron concentration. The differences in iron concentration (Δ) are compared against the mean [Fe] dry weight for a reference (ref) region. American Heart Association segments are numbered and labeled as follows: ant, anterior; sep, septum; lat, lateral; and inf, inferior.
iron in the present study. Our statistical analysis demonstrated that this relationship was curvilinear. Previous investigators have described a linear relationship between R2* and iron concentration, but these studies had fewer observations and a more limited range of measurement.17 Although R2* underestimation may occur at high iron concentrations if initial echo times are too long, a minimum echo time of 1.2 milliseconds should have been adequate to capture even the most heavily loaded samples. In addition, we found little calibration differences if heart 4 was included or excluded. For clinical purposes, for which T2* is typically the result of interest, tolerance to myocardial iron can only be speculative, but may have been genetically mediated.

Also of interest is the myocardial T2* value in relation to iron levels commonly seen in the liver in β-thalassemia major, indicating that the heart is considerably more sensitive to iron loading than the liver in terms of functional consequences. The outlier heart had 5 times the iron concentration compared with the mean value of the other hearts, functional consequences. The outlier heart had 5 times the iron concentration compared with the mean value of the other hearts, which equates to 0.50 mg/g dw. These values accord well with the previous published normal values for myocardial T2* and Cardiac Iron 1525

Table 3. Distribution of Iron in the Right Ventricle, Atria, and Valves

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>All Hearts (Excluding Heart 4)</th>
<th>Heart 4 Alone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Fe], mg/g</td>
<td>Lower 95% CI</td>
</tr>
<tr>
<td>Global LV [Fe] (reference)</td>
<td>5.98</td>
<td>4.52</td>
</tr>
<tr>
<td>RV Posterior (Δ)</td>
<td>-1.26</td>
<td>-1.75</td>
</tr>
<tr>
<td>RV Anterior (Δ)</td>
<td>-1.31</td>
<td>-1.80</td>
</tr>
<tr>
<td>Apical (Δ)</td>
<td>-0.98</td>
<td>-1.57</td>
</tr>
<tr>
<td>Mid (Δ)</td>
<td>-1.43</td>
<td>-2.02</td>
</tr>
<tr>
<td>Basal (Δ)</td>
<td>-1.45</td>
<td>-2.04</td>
</tr>
<tr>
<td>Atria and valves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global LV [Fe] (reference)</td>
<td>5.98</td>
<td>4.57</td>
</tr>
<tr>
<td>LA anterior (Δ)</td>
<td>-0.78</td>
<td>-1.64</td>
</tr>
<tr>
<td>LA posterior (Δ)</td>
<td>-1.68</td>
<td>-2.59</td>
</tr>
<tr>
<td>LA lateral (Δ)</td>
<td>-1.11</td>
<td>-1.98</td>
</tr>
<tr>
<td>Atrial septum (Δ)</td>
<td>-2.99</td>
<td>-3.86</td>
</tr>
<tr>
<td>RA anterior (Δ)</td>
<td>-3.28</td>
<td>-4.15</td>
</tr>
<tr>
<td>RA posterior (Δ)</td>
<td>-3.41</td>
<td>-4.28</td>
</tr>
<tr>
<td>RA lateral (Δ)</td>
<td>-3.09</td>
<td>-3.95</td>
</tr>
<tr>
<td>Aortic valve (Δ)</td>
<td>-4.45</td>
<td>-5.57</td>
</tr>
<tr>
<td>Mitral valve (Δ)</td>
<td>-4.37</td>
<td>-5.23</td>
</tr>
<tr>
<td>Pulmonary valve (Δ)</td>
<td>-4.33</td>
<td>-5.45</td>
</tr>
<tr>
<td>Tricuspid valve (Δ)</td>
<td>-4.40</td>
<td>-5.26</td>
</tr>
</tbody>
</table>

[Fe] indicates myocardial iron concentration; CI, confidence interval; RV, right ventricle; LV, left ventricle; Ref, reference; LA, left atrial; and RA, right atrial. The differences in iron concentration (Δ) are compared against the mean LV [Fe] dry weight.
iron and with the global myocardial T2* value (44.4 milliseconds) of 1 patient in our study who died of a stroke. The myocardial T2* values associated with being below the normal range are typically /H1102120 milliseconds (1.1 mg/g dw), and as T2* falls below 10 milliseconds (2.7 mg/g dw), there is a progressive decline in both LV and RV ejection fractions.8,34 The vast majority of patients who present with heart failure caused by cardiac iron overload have T2* /H1102110 milliseconds, and low T2* values are powerful independent predictors of the subsequent development of cardiac failure.35 All but one of the hearts in this study were from patients who either had died of heart failure or had undergone cardiac transplantation for end-stage heart failure. In keeping with clinical experience, the mean myocardial T2* was 5.9±3.0 milliseconds (range, 2.0 to 12.3 milliseconds).

Limitations
This project has required international collaboration between specialist centers and could not have been performed with fresh, unfixed hearts, owing to regulations on transport of human tissue between sites. The fact that death resulting from cardiac failure is almost always restricted to patients with severe cardiac iron loading (T2* <10 milliseconds) is reflected in the range of T2* values. Although there is no guarantee that in vivo myocardial T2* and ex vivo myocardial T2* are the same, the data are supportive with good agreement between premortem and postmortem T2* values in 3 hearts. Some hearts had been stored in formalin over extended periods, but our data suggest that there is no significant change in T2* over time and only a small amount of leaching of iron into the formalin solution. Although every effort was made to correlate ROIs used for R2* measurement with the tissue blocks analyzed for iron concentration, it is not possible to eliminate disparity completely There are difficulties with R2* measurement in heavily iron-loaded tissue in which signal decay is rapid, requiring very short echo times. Scanner hardware imposes constraints on minimum echo spacing, which may be inadequate for accurate R2* measure-
ment at very high iron concentrations. However, a minimum echo time of 1.2 milliseconds should allow reliable measurement of T2* values as low as 1.44 milliseconds,16 which is below the minimum global myocardial value seen in this study. Finally, some ROIs were excluded from the calibration because of imaging artifact. The decision to exclude an ROI was made by subjective assessment before the analysis of R2* and is unlikely to have affected the calibration significantly, owing to the many regions sampled throughout the 12 hearts. Previous reports have examined the myocardial relaxation parameter T2 in relation to cardiac iron in animal models16 and in humans.16,19 T2 imaging can be challenging, however, and it is not in routine clinical use, although recent imaging advances have been made.37 Because of the ease of acquisition and robust nature of T2* for clinical practice, we have concentrated on this technique.

Figure 6. Septal cardiac magnetic resonance measurements vs myocardial iron concentration. A, Mean septal R2* plotted against mean iron concentration for each heart. Error bars are ± SD for septal R2*, and the regression line is based on the log-log data in Figure 5E. B, Mean septal T2* plotted against mean iron concentration for each heart. These 2 graphs illustrate the difference between T2* and R2* compared with tissue iron. T2* shortens with increased iron concentration; hence, its reciprocal, R2*, rises with tissue iron. T2* is used for clinical assessment of cardiac iron for historical reasons.

Conclusion

The results of the present study validate the clinical technique in current use and provide detailed calibration and distribution data in humans.

Acknowledgments

We are indebted to the generosity of the patients and families without whose help we would have been unable to perform this important study. We would like to thank Steven Collins for assistance in scanning the ex vivo hearts and Cathleen Enrizquez for her assistance in preparing this manuscript.

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Disclosures

Dr Carpenter has received honoraria from Novartis, Apotex, and Swedish Orphan. Dr He is supported by the British Heart Foundation and is a consultant to Novartis. Dr Porter has received research funding from and served on the speakers’ bureau and advisory board for Novartis. Professor Galanello served on the speakers’ bureau for Novartis and ApoPharma. Dr Forni has received research funding from Novartis. Dr Fuchareon is a Senior Research Scholar of the Thailand Research Fund. Dr Firmin has received research support from Siemens. Dr Pennell is a consultant to and has served on advisory boards and speakers’ bureaus for Novartis, ApoPharma, and Siemens; has received research funding from Novartis; and is a director and stockholder for Cardiovascular Imaging Solutions. Dr St. Pierre holds shares in and is on the Board of Directors of Resonance Health Ltd and has received research funding from Novartis. Dr Wood has received research funding from Novartis and is a consultant for Ferrokin Biosciences. The other authors report no conflicts.

References

many thousands of transfusion-dependent patients worldwide. Iron accumulation leads to damage in the liver, heart, and endocrine organs.

Cardiac iron overload is a major killer in transfusion-dependent patients and others with errors of iron metabolism. This applies especially to the large iron overload centers.


A strong correlation was observed between cardiovascular magnetic resonance against human iron concentration and the iron distribution throughout the heart under conditions of iron overload. A single breath-hold multiecho T2* cardiovascular magnetic resonance technique for diagnosis of myocardial iron overload. J Magn Reson Imaging. 2003;18:33–39.

The data also show that cardiovascular magnetic resonance measurements can be used to measure myocardial iron and validate its use as an end point in clinical trials for the improvement of efficacy of cardiac iron chelation treatment to prevent heart failure and death. This will significantly affect the health care of the many thousands of transfusion-dependent patients worldwide.
On T2* Magnetic Resonance and Cardiac Iron

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