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Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload

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Aims To develop and validate a non-invasive method for measuring myocardial iron in order to allow diagnosis and treatment before overt cardiomyopathy and failure develops.

Methods and Results We have developed a new magnetic resonance T2-star (T2*) technique for the measurement of tissue iron, with validation to chemical estimation of iron in patients undergoing liver biopsy. To assess the clinical value of this technique, we subsequently correlated myocardial iron measured by this T2* technique with ventricular function in 106 patients with thalassaemia major. There was a significant, curvilinear, inverse correlation between iron concentration by biopsy and liver T2* ($r=0{\cdot}93$, $P<0{\cdot}0001$). Inter-study cardiac reproducibility was 5·0%. As myocardial iron increased, there was a progressive decline in ejection fraction ($r=0{\cdot}61$, $P<0{\cdot}001$). All patients with ventricular dysfunction had a myocardial T2* of <20 ms. There was no significant correlation between myocardial T2* and the conventional parameters of iron status, serum ferritin and liver iron. Multivariate analysis of clinical parameters to predict the requirement for cardiac medication identified myocardial T2* as the most significant variable (odds ratio 0·79, $P<0{\cdot}002$).

Conclusions Myocardial iron deposition can be reproducibly quantified using myocardial T2* and this is the most significant variable for predicting the need for ventricular dysfunction treatment. Myocardial iron content cannot be predicted from serum ferritin or liver iron, and conventional assessments of cardiac function can only detect those with advanced disease. Early intensification of iron chelation therapy, guided by this technique, should reduce mortality from this reversible cardiomyopathy.
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Key Words: Cardiomyopathy, magnetic resonance imaging, heart failure and thalassaemia.

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Introduction

Cardiac failure secondary to transfusional iron overload remains the commonest cause of death in patients with thalassaemia major[1,2]. In the United Kingdom, approximately 50% of patients die before reaching the age of 35[3]. The cardiomyopathy is reversible if intensive iron chelation treatment is instituted in time[4–6], but diagnosis is often delayed by the unpredictability of cardiac iron deposition and the late development of symptoms, and echocardiographic abnormalities[7,8]. Once heart failure develops, the outlook is usually poor[9] with precipitous deterioration and death, despite intensive chelation. Direct measurement of myocardial iron would allow earlier diagnosis and treatment and help to reduce mortality from this reversible cardiomyopathy.

The aim of this study was to develop a reproducible magnetic resonance (MR) method for quantifying myocardial iron concentration. For this purpose, we investigated myocardial T2-star (T2*), a relaxation parameter arising principally from local magnetic field inhomogeneities that are increased with iron deposition.

Although a rare disease in the U.K., thalassaemia is the commonest genetic disorder worldwide, with approximately 94 million heterozygotes for beta thalassaemia and 60 000 homozygotes born each year[10]. Iron overload cardiomyopathy is also a complication of hereditary haemochromatosis, which predominantly

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affects those of northern European ancestry, where homozygous mutations of the HFE gene approximate 0·5%[11]. Patients receiving multiple transfusions during chemotherapy and bone marrow transplant, or for other indications such as sickle cell anaemia, may also benefit from assessment with this technique.

Methods

Study populations

Liver biopsy patients

We prospectively studied 30 beta-thalassaemic patients (12 females and 18 males, aged 18–38, mean 27·1 ± 6·7 years) undergoing liver biopsy for routine clinical management. The biopsy iron concentrations were compared with the liver T2* measurements derived by MR. All scans were performed within 21 days of the liver biopsy (mean 10 ± 7·0 days) and no adjustments to chelation treatment were made between investigations. In 27 cases, a section of the biopsy specimen was also examined histologically (cirrhosis 3 patients, periportal fibrosis 10 patients).

Thalassaemia major cohort

A total of 109 regularly transfused patients with thalassaemia major were scanned. Three patients were excluded from comparison analysis of ventricular function due to cardiac anomalies (1 corrected tetralogy of Fallot, 1 subaortic shelf and 1 peripheral pulmonary artery stenosis). The residual cohort of 106 patients included 50 males and 56 females, aged 13–41, mean 27 ± 7 years. All patients had received iron chelation therapy since the mid-to-late 1970s, or from early childhood in patients born after this time, with a broad range of compliance to treatment (serum ferritin 262–7624 $\mu g \cdot l^{-1}$, mean $2095 \pm 1559\ \mu g \cdot l^{-1}$). Seventeen patients required medication for ventricular dysfunction (antiarrhythmics or angiotensin-converting-enzyme inhibitors).

Normal subjects

Normal ranges for T2* values in the liver, heart, spleen and skeletal muscle were established in 15 healthy volunteers (9 males, 6 females, aged 26–39, mean 31 ± 3·7 years).

Liver biopsy iron assays

All biopsy specimens were analysed at the Royal Free Hospital, London[12]. The dry weights of all specimens in this study exceeded 0·5 mg (mean 1·33 ± 0·59 mg).

Serum ferritin measurements

Measurements of serum ferritin were carried out by enzyme immunoassay (WHO Ferritin 80/602 First International Standard, normal range 15–300 $\mu g \cdot l^{-1}$).

Magnetic resonance

Patients were scanned with a Picker 1·5T Edge Scanner (Marconi Medical Systems, Ohio, U.S.A.). Each scan lasted approximately 45 min and included the measurement of liver and heart T2*, and left and right ventricular function, volumes and mass using standard techniques[13].

The liver T2* was determined as follows: a single 10 mm slice through the centre of the liver was scanned at eight different echo times (TE 2·2–20·1 ms). Each image was acquired during a 10–13 s breath-hold using a gradient-echo sequence (repetition time 200 ms, flip angle 20°, matrix 96 × 128 pixels, field of view 35 cm, sampling bandwidth of 125 kHz). The signal intensity of the liver parenchyma and the background noise were measured in each of the eight images using in-house software (CMRtools, © Imperial College). Background noise was subtracted from the liver signal intensity, and the net value was plotted against the echo time for each image. A trendline was fitted to the resulting exponential decay curve, with an equation of the form $y = Ke^{-TE/T2^*}$ where K represents a constant, TE represents the echo time and y represents the image signal intensity.

For the measurement of myocardial T2*, a single short axis mid-ventricular slice was acquired at nine separate echo times (TE 5·6–17·6 ms). The repetition time between radiofrequency pulses was between 11·8–23·8 ms, depending on the echo time used. A gradient–echo sequence was used (flip angle 35°, matrix 128 × 256 pixels, phase encode group 8, field of view 35 cm, sampling bandwidth of 250 kHz). The repetition time was adjusted to the patient's heart rate. Each image was acquired during an 8–13 s breath-hold. A gating delay time of 0 ms after the R-wave was chosen in order to obtain myocardial images in a consistent position in the cardiac cycle irrespective of the heart rate. A full-thickness region of interest was measured in the left ventricular myocardium, encompassing both epicardial and endocardial regions. This was located in the septum, distant from the cardiac veins, which can cause susceptibility artefacts[14]. The myocardial T2* was calculated using the same method as that in the liver.

Statistical analysis

Summary data are presented as mean ± 1 standard deviation. Pearson's and Spearman's tests were used to assess the correlation between liver iron and liver T2*. For reproducibility data, the coefficient of variation was defined as the standard deviation of the differences between the two separate measurements, divided by their mean and expressed as a percentage. T2* values measured in healthy volunteers showed a normal distribution and are expressed with 95% reference ranges. Pearson's coefficient of correlation was used to assess the degree of association between myocardial T2* and liver

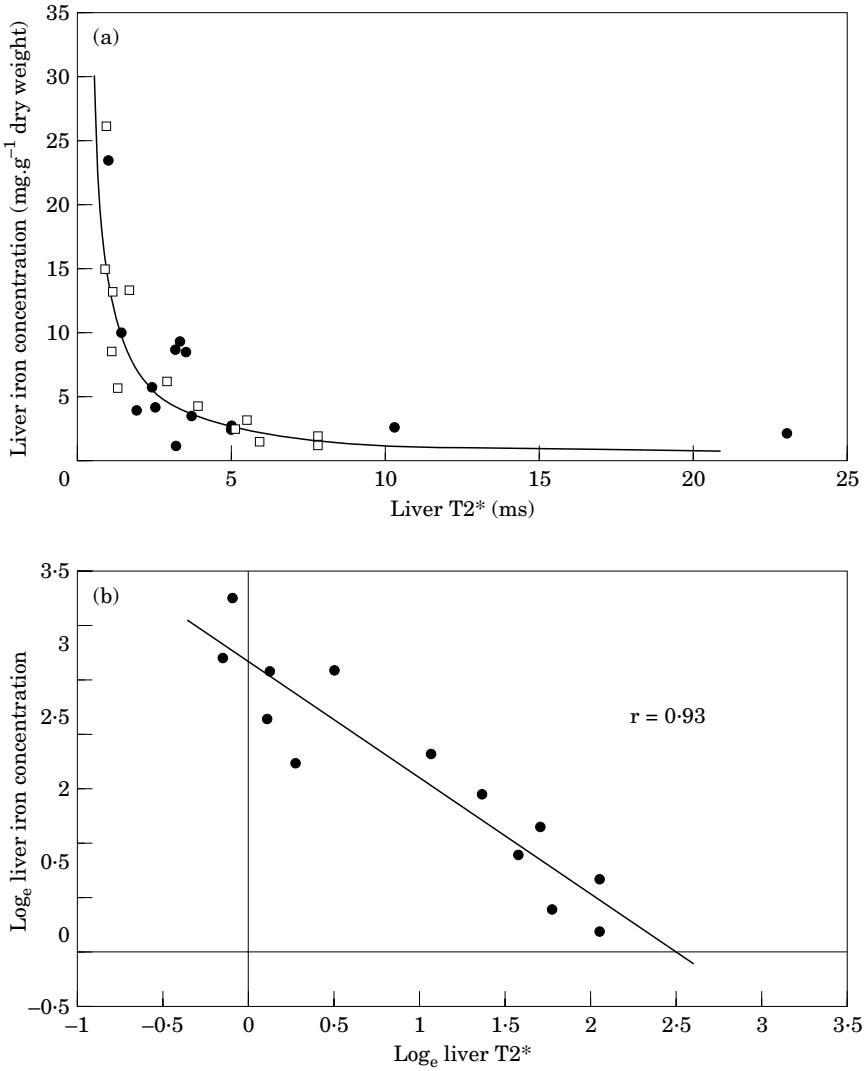

Figure 1 **(a) Regression curve for the relationship between liver T2* and liver biopsy iron concentration. Black circles depict fibrotic biopsies, and squares depict non-fibrotic biopsies. The fibrotic samples show increased variability, compatible with previous reports. (b) There was a close linear relation between T2* and liver iron concentration in the non-fibrotic samples following log$_e$ transformation (r=0·93, *P*<0·0001), see text for details.**

T2* and myocardial T2* and serum ferritin. Stata statistical software was used for computations (Stata Corporation, Texas, U.S.A.).

Results

Validation of T2 values as a measurement of tissue iron concentration*

There was a significant, curvilinear, inverse correlation between liver T2* and the liver iron content for all samples (r=0·81, Fig. 1(a)). There was a better correlation with the non-fibrotic liver samples (r=0·93) than the fibrotic samples (r=0·68), as would be predicted from the known variability of iron measurements from fibrotic biopsies[15,16]. Therefore we subsequently employed non-fibrotic samples to generate predictions of liver iron content from the measured T2* values. As liver iron concentration and liver T2* measurements were positively skewed, the values were log$_e$ transformed in order to analyse the correlation (Fig. 1(b)). For the non-fibrotic samples, both Pearson's and Spearman's tests gave a correlation coefficient of 0·93 which is highly significant (*P*<0·0001). Regression analysis shows that a one unit increase in log$_e$ T2* is associated with a 1·07 unit increase in log$_e$ iron concentration (95% confidence interval 0·78 to 1·35 unit decrease).

Reproducibility

Ten patients were scanned on two occasions to assess the inter-study reproducibility of the T2* technique (interval 1–21 days, mean 7·1 days). The coefficient of variation was 3·3% for the liver and 5·0% for the heart. This compared favourably with coefficients of variation for signal intensity ratio measurements from these same images (liver-to-muscle 7·9%, liver-to-noise 8·8%, heart-to-muscle 12·6%, and heart-to-noise 14·1%), techniques that have previously been used.

The images from 10 patients were studied independently by two observers to assess inter-observer variability. The coefficient of variation was 4·5% for the liver and 6·4% for the heart. This compared favourably with signal intensity ratio measurements (liver-to-muscle 5·4%, liver-to-noise 6·1%, heart-to-muscle 10·8%, and heart-to-noise 7·5%).

Normal T2 values*

The normal values for T2* using the technique described above were: Heart 52 ± 16 ms, liver 33 ± 7 ms, skeletal muscle 30 ± 5 ms, spleen 56 ± 22 ms.

Heart iron, liver iron, serum ferritin and the relationships between these variables

In many patients we found a marked discordance between liver and heart iron concentration (Fig. 2) and no significant correlation could be found between liver and heart T2* in this large cohort ($r=0·15$, $P=0·11$). Similarly, no significant correlation was found between heart T2* and serum ferritin level at the time of the scan ($r=0·10$, $P=0·32$). To confirm that this finding was not due to spurious individual ferritin readings, the mean ferritin for 12 months prior to the scan was also compared to heart T2*, and once again there was no significant correlation ($r=0·09$, $P=0·35$).

Myocardial iron and parameters of ventricular function

In the normal range of myocardial T2* (lower 95% confidence interval 20 ms), parameters of ventricular function (ejection fraction, volume and mass) fell within the normal range[17] (Fig. 3). Below a myocardial T2* of 20 ms, there was a progressive and significant decline in left ventricular ejection fraction ($r=0·61$, $P<0·0001$) and an increase in the left ventricular end-systolic volume index ($r=0·50$, $P<0·0001$), and left ventricular mass index ($r=0·40$, $P<0·001$).

Myocardial T2 and clinical outcome*

Logistic regression was performed to relate the requirement for cardiac medication to seven clinical covariates.

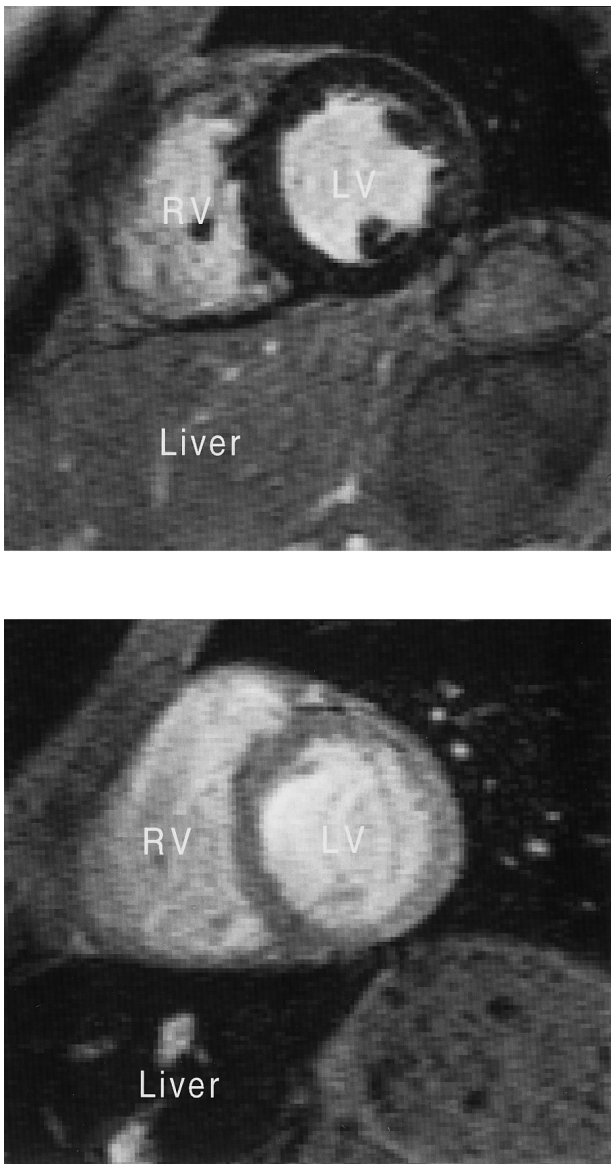

Figure 2 **Discordance of liver and heart iron deposition. Short axis plane, including the adjacent liver (TE 5·6 ms). The top panel shows a patient with severe cardiac iron deposition but minimal liver iron deposition (heart darker than liver). The lower panel shows a patient with normal myocardial iron but severe liver iron overload (liver darker than heart).**

Of 106 patients, 17 patients required medication for ventricular dysfunction, and univariate analysis identified myocardial T2*, left ventricular ejection fraction and left ventricular end systolic volume as significant variables (Table 1). Using multivariate backward stepwise regression analysis, with a cut-off of $P=0·1$ for removing variables and $P=0·05$ for including variables, only myocardial T2* (odds ratio 0·79, 95% confidence interval 0·67–0·92, $P=0·002$) and serum ferritin (odds ratio 0·95, 95% confidence interval 0·91–1·00, $P=0·05$) were significant. Depite the lack of correlation between myocardial T2* and serum ferritin, both are predictors

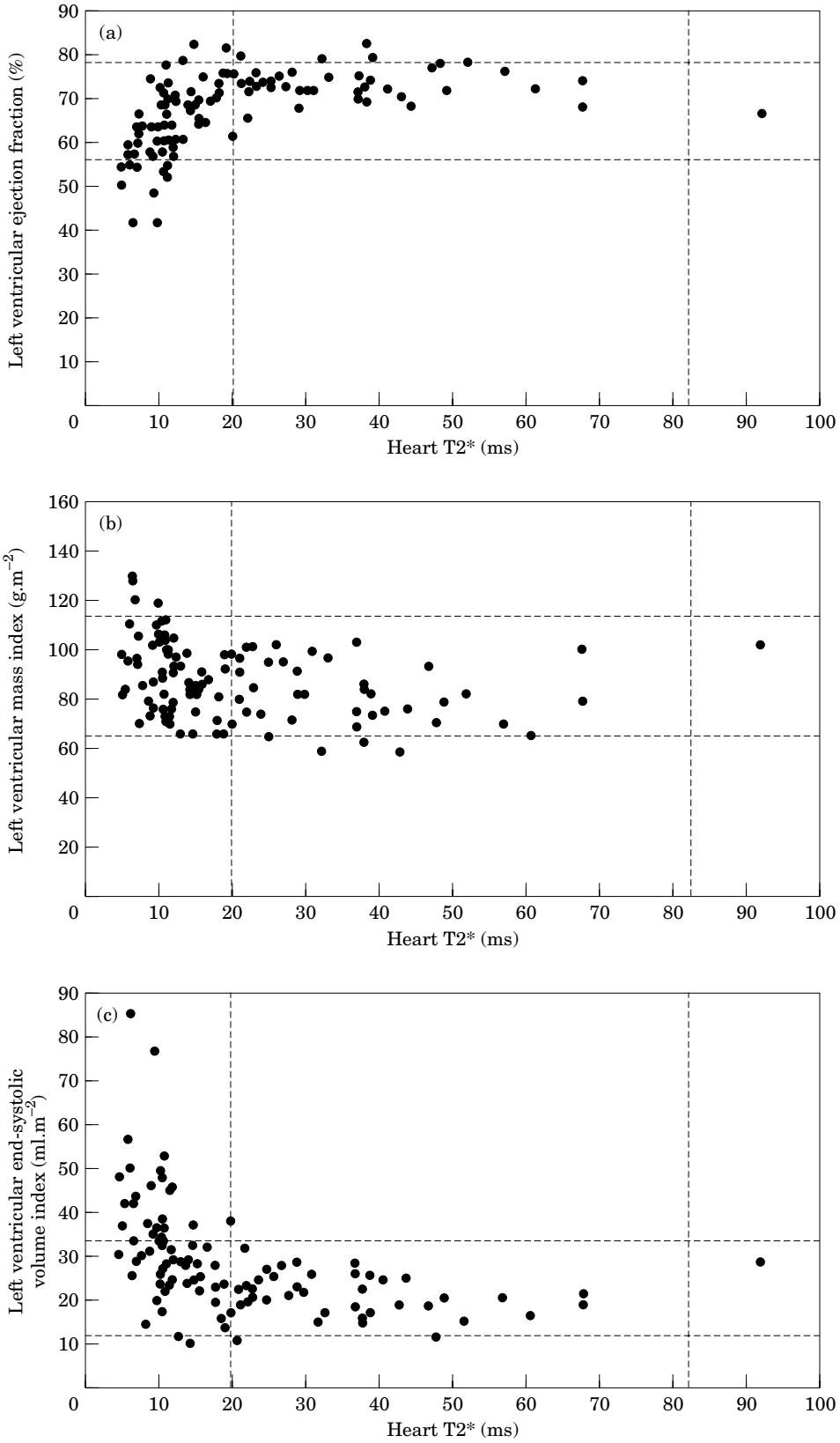

Figure 3 **Relationships between myocardial T2* values and parameters of ventricular function: (a) left ventricular ejection fraction, (b) left ventricular mass index, (c) left ventricular end-systolic volume index. The broken lines represent the normal reference ranges for myocardial T2* and parameters of cardiac function.**

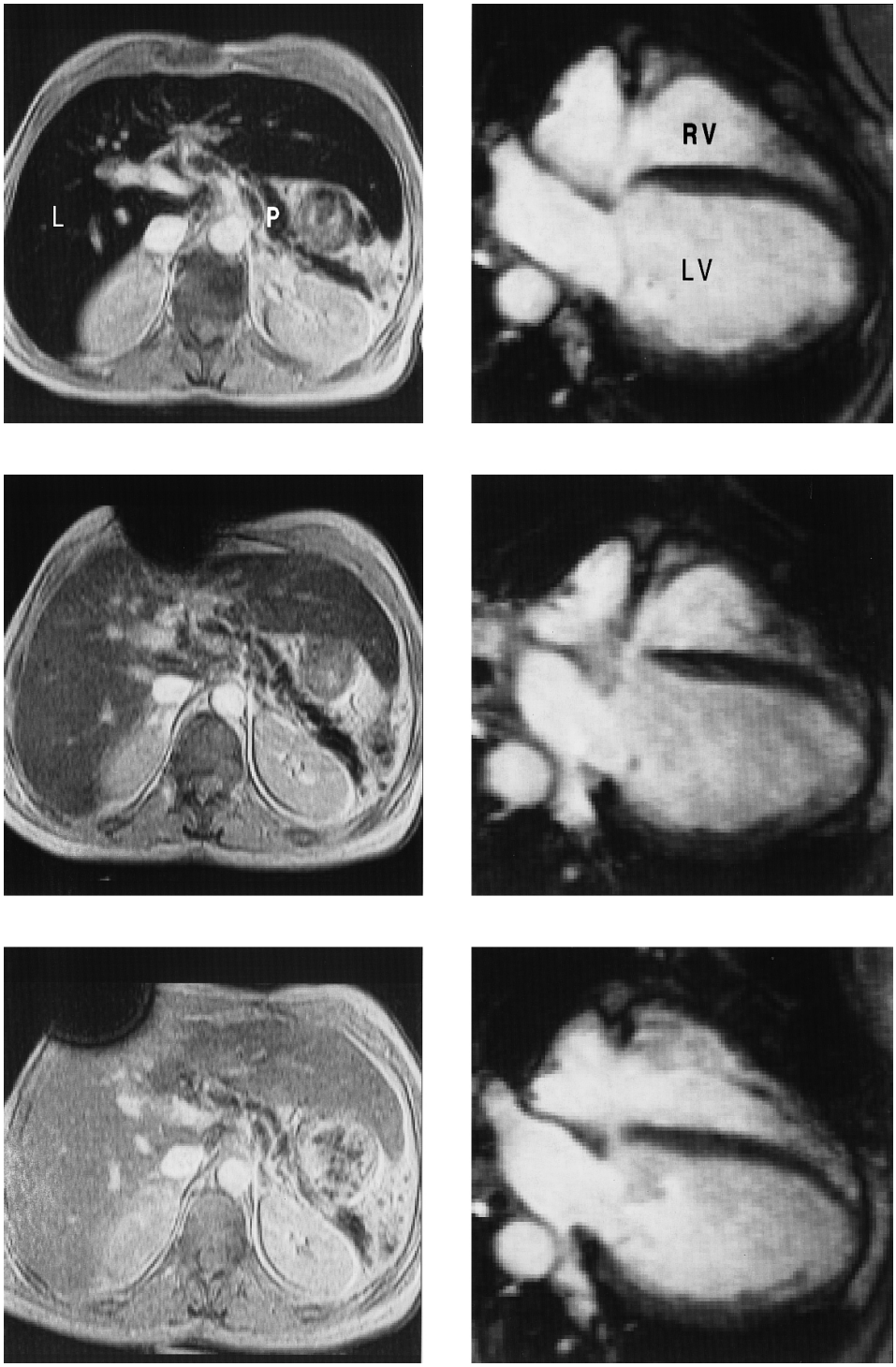

Figure 4 **MR gradient echo images of differential tissue iron clearance before and during intravenous chelation therapy: before treatment (top row), after 3 months (middle row) and after 6 months of treatment (bottom row). The left column shows the liver (transaxial view, TE 4·5 ms), and the right column shows the heart (horizontal long axis, TE 14 ms), which is dilated (RV — right ventricle 182 ml end-diastolic volume, LV — left ventricle 183 ml end diastolic volume). There is severe iron loading (dark tissue signal) in the liver (L), pancreas (P) and heart prior to treatment (liver T2* = 1·2 ms, myocardial T2* = 10 ms). By 3 months, the liver iron is noticeably improved (liver T2* = 5·1 ms), but cardiac iron deposition has changed little (T2* = 10·1 ms). Myocardial iron deposition only shows improvement at 6 months (T2* = 12·1 ms) and even at this time the heart remains dilated (end-diastolic volume = 176 ml). The liver at this time, however, has nearly normalized (T2* 8·1 ms).**

for cardiac medication. This may be related to increased clinical vigilance in the treatment of patients with high serum ferritins. As cardiac medication had been initiated in all patients prior to CMR scanning, physicians were blinded to myocardial T2* measurement. Myocardial T2* values were between 4·9 ms and 13 ms in this group.

Discussion

Iron overload pathophysiology

Iron overload occurs either due to excess gastrointestinal absorption or secondary to repeated blood transfusions. The human body has no mechanism for excreting excess iron, which is stored as crystalline iron oxide within ferritin and haemosiderin in the body. The aetiology of the iron overload effects the tissue distribution of iron. In hereditary haemochromatosis, iron is carried from the intestine to the liver via the portal vein (as transferrin) and deposited in the periportal hepatocytes. In severe disease, iron is also deposited in the pancreas, heart and endocrine organs. In thalassaemia, iron overload results from both excessive iron absorption and transfusional siderosis. Transfusional iron leads to iron deposition in the reticulo-endothelial system of the spleen, liver and bone marrow. In advanced cases iron also accumulates in parenchymal cells of the liver, heart, pancreas and endocrine organs, which are sensitive to the toxic effects of iron. When the iron-binding capacity of transferrin is exhausted, free iron appears as non-transferrin bound iron (NTBI). The toxicity of NTBI is much higher than bound iron, and promotes hydroxyl radical formation resulting in peroxidative damage to membrane lipids and proteins. In the heart this results in impaired function of the mitochondrial respiratory chain and is manifested clinically as heart failure[18].

The presence of two types of iron explains the nature of the relationship between myocardial function and iron concentration (as shown in Fig. 3). As iron accumulates in the normal storage form in the heart, the T2* falls, but there is little effect on cardiac function until a threshold is reached where the iron storage capacity is exhausted. At this point NTBI starts to appear, which profoundly affects cardiac function. Thus the relationship between the measured T2* and cardiac function is shallow until a critical level is reached, after which rapid deterioration occurs. This explains why identification of abnormal systolic function is a late sign of iron toxicity. Iron clears more slowly from the heart than the liver (Fig. 4), which may contribute to the high mortality of patients with established cardiomyopathy despite intensive chelation. Using this T2* technique, it is possible to identify much earlier those patients who require intensive chelation prior to the onset of systolic dysfunction and this should avoid the mortality associated with overt heart failure.

Table 1 Univariate analysis of clinical variables to test the strength of their relationship to the need for cardiac medication

Variable	Odds ratio (95% CI)	*P* value
Myocardial T2* (ms)	0·81 (0·71, 0·93)	0·003
LVEF (%)	0·88 (0·82, 0·94)	<0·001
LVESV (ml)	1·05 (1·02, 1·08)	0·001
Serum ferritin ($\mu g \,.\, l^{-1}$)	0·97 (0·93, 1·01)	0·17
Liver T2* (ml)	1·01 (0·91, 1·12)	0·85
Diabetes mellitus	1·58 (0·56, 4·51)	0·39
Age	1·01 (0·94, 1·08)	0·85

CI=confidence interval; LVEF=left ventricular ejection fraction; LVESV=left ventricular end systolic volume.

MR T2 technique to measure myocardial iron*

We chose a gradient-echo T2* sequence rather than a spin-echo T2 sequence because of the greater sensitivity to iron deposition. T2* is related to T2 by summation of tissue relaxation (T2), and magnetic inhomogeneity, known as T2 prime (T2′), in the form:

$$1/T2^* = 1/T2 + 1/T2'$$

Iron overload causes signal loss in affected tissues because iron deposits become magnetized in the scanner, inducing local irregularities in the magnetic field, which cause water protons around these deposits to lose phase coherence[19]. This effect is concentration dependent[20]. An additional benefit of the shorter acquisition times of gradient-echo images is minimization of motion artefacts from myocardial contraction and respiratory movement, which greatly affect the accuracy and reproducibility of T2 images.

Previous work using spin-echo techniques with signal-intensity-ratios or T2 measurements, have shown an inverse relationship to liver iron concentration[21–24]. However, in practice, the limited sensitivity of spin-echo techniques, motion artefacts and poor signal to noise at longer echo times[21,22,25], have made quantification of myocardial iron unsatisfactory[26]. Because of these problems, gradient-echo techniques using signal-intensity-ratios have recently been used to quantify liver iron[27–29], but no studies in the heart have been reported. We have used multiple echoes to generate T2* instead of relying on signal ratios between tissues, and for the first time have applied the technique to the heart. This range of echo times improves quantification of severe iron overload, provides high sensitivity at low and normal tissue iron levels, and gives greater reproducibility than signal intensity ratios.

The normal value of myocardial T2* in this study was 52 ± 16 ms. There is limited literature with which to compare these results. Li *et al.* studied 13 normals and reported a T2* of $33 \pm 6{\cdot}5$ ms, but only two echo times were used[30]. Wacker *et al.* reported the normal myocardial value in six patients with coronary disease

(remote from ischaemia) as 48 ± 9 ms using a 10 echo time technique[31]. Reeder reports normal T2* values of 38 ± 6 ms in the mid septum in five normal volunteers, and showed reduced values adjacent to the cardiac veins due to their local susceptibility[14]. The variation in these values may result from residual T1 effects associated with the short repetition times imposed in breath-hold acquisitions, and may lead to over-estimation of T2*. However, this effect is much less significant in the presence of short myocardial T2* values in iron overload.

Validation of T2 measurements, and variability between tissues*

We have shown a significant curvilinear correlation between liver T2* and biopsy iron concentration ($r=0{\cdot}93$, $P<0{\cdot}0001$, for non-fibrotic livers). Using the T2* technique in a large cohort of patients, we also found that there is no reliable relationship between myocardial T2* and serum ferritin or liver T2*. This indicates that cardiological management based on these established parameters of iron status is unreliable.

Heart failure and causality

The poor predictive value of serum ferritin and liver iron measurements have made heart disease difficult to detect in thalassaemia, raising questions over the causal relationship between cardiac iron overload and cardiac failure[32–34]. Recently, myocarditis has been implicated in the development of heart failure in thalassaemia[35,36]. This study demonstrates the relationship between deterioration in ventricular function and myocardial iron loading and illustrates clear evidence for the causality of iron overload and heart failure in thalassaemia patients. Whilst our study supports the aetiological role of iron in thalassaemic cardiomyopathy, other factors such as antioxidant state[37] may also be important.

Study limitations

It is not possible definitively to predict myocardial iron concentration from the myocardial T2* value, because no validation has been performed with cardiac tissue. This requires myocardial biopsies and will be difficult because of inhomogenous myocardial deposition[33,38] and small samples. Nonetheless, the data presented in this study, showing the strong relationship between declining myocardial T2* and impaired ventricular function, clearly indicates the empirical value of myocardial T2*, and the validation data from the liver biopsies supports the relationship between tissue iron and T2*.

The T2* of iron-loaded tissue decreases with increasing field strength and therefore the threshold of T2*<20 ms is applicable only to 1·5T scanners. In addition, relaxation parameters such as T2* may be machine and sequence dependent, and further validation work is required before widespread use.

Conclusions

Gradient-echo T2* MR provides a rapid, non-invasive, reproducible means for assessing myocardial iron. Myocardial iron content cannot be predicted from serum ferritin or liver iron, and conventional assessments of cardiac function can only detect those with advanced disease. Early diagnosis and treatment of myocardial iron overload is likely to prevent the mortality seen in patients with established ventricular dysfunction.

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