Original Research

Normalized Left Ventricular Volumes and Function in Thalassemia Major Patients With Normal Myocardial Iron

Mark A. Westwood, MD,[1] Lisa J. Anderson, MD,[1] Alicia M. Maceira, MD,[1] Farrukh T. Shah, MBBS,[2] Emma Prescott, BSc,[3] John B. Porter, PhD,[3] Beatrix Wonke, MD,[3] J. Malcolm Walker, MD,[3] and Dudley J. Pennell, MD[1]*

Purpose: To determine the reference range in thalassemia major (TM) for left ventricular (LV) function.

Materials and Methods: We used cardiovascular magnetic resonance (CMR) to measure heart volumes and function in 81 TM patients with normal myocardial T2* measurements (T2* > 20 msec) and by inference without excess myocardial iron. Forty age- and gender-matched healthy controls were also studied.

Results: Resting LV volumes and function normalized to body surface area differed significantly between TM patients and controls. The lower limit and the mean for ejection fraction (EF) were higher in TM patients (males 59 vs. 55%, mean 71% vs. 65%; females 63 vs. 59%, mean 71% vs. 67%; both $P < 0.001$). The upper limit and mean for end-diastolic volume index were higher in TM patients (males 152 vs. 105 mL/m^2, mean 97 vs. 84 mL/m^2; females 121 vs. 99 mL/m^2, mean 87 vs. 79 mL/m^2; both $P < 0.05$). In TM patients the cardiac index ($P < 0.001$) was increased.

Conclusion: At rest, TM patients with a normal myocardial T2* have different "normal" values for LV volume and function parameters compared to controls, and this has the potential to lead to a misdiagnosis of cardiomyopathy. We present new reference "normal" ranges in TM to alleviate this problem.

Key Words: heart; iron; magnetic resonance; heart function; heart failure
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THE THALASSEMIAS ARE THE MOST COMMON inherited single gene disorders worldwide, and result in reduced or absent transcription of either the alpha or the beta chains of hemoglobin. Beta thalassemia major (TM) results from the absence of or a marked decrease in beta chain synthesis and requires regular blood transfusions from birth to prolong life (1). However, this leads to an inexorable accumulation of iron in the tissues and to iron-induced tissue injury. Cardiomyopathy is reported to develop at a mean age of 16 years without treatment (2) and accounts for the majority of all TM deaths. It has been reported that only 50% of UK patients survive beyond the age of 35 years (3); however, more recent cohorts have shown improved survival (4). Both serum ferritin measurements (5–8) and liver biopsy estimations (9–11) have limitations in the assessment of myocardial iron, and recent reports have shown a poor correlation between myocardial T2* with hepatic T2* in a cross section of patients (12) and in patients receiving different chelation regimes (13).

One means of detecting myocardial iron toxicity that has been widely applied is the serial follow-up of left ventricular (LV) volume and function by echocardiography (echo) or radionuclide ventriculography (4,14). LV parameters in TM patients are compared with established ranges from normal non-anemic subjects. However, this is subject to three separate and opposing influences that may make such a comparison unreliable for detecting possible early cardiomyopathy: 1) some TM patients may have an atypical body habitus due to growth retardation that results in lower absolute LV measurements; 2) the degree of chronic anemia may vary depending on the transfusion regimen adopted, which may increase the cardiac output and dimensions (15); and 3) preexisting iron overload cardiomyopathy may affect ventricular size. Therefore, LV dilatation may be difficult to interpret (16).

Cardiovascular magnetic resonance (CMR) is in a unique position to tease out these influences and determine the normal range of LV size and function in TM. This is because in addition to quantifying parameters of LV size and function in an accurate (17) and highly reproducible way (18), with this approach one can in-

[1]Cardiovascular MR Unit, Royal Brompton Hospital and Imperial College, London, United Kingdom.
[2]Haematology Department, Whittington Hospital, London, United Kingdom.
[3]Haematology Department, University College Hospital, London, United Kingdom.
Contract grant sponsors: CORDA; Wellcome Trust; British Heart Foundation.
*Address reprint requests to: D.P., Cardiovascular MR Unit, Royal Brompton Hospital, Sydney Street, London SW3 6NP, UK.
E-mail: d.pennell@ic.ac.uk
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Table 1
Comparison of Demographics of TM Patients and Controls

	TM patients[a]	Controls[a]	*P* value
Males			
Age (years)	28 ± 7	30 ± 4	0.41
Height (m)	1.6 ± 0.1	1.8 ± 0.1	<0.001
Weight (kg)	64.8 ± 10.2	75.8 ± 10.0	<0.001
BMI (kg/m^2)	24.4 ± 3.3	23.6 ± 3.1	0.39
BSA (m^2)	1.7 ± 0.2	1.9 ± 0.1	<0.001
Heart rate (per minute)	81.7 ± 16.4	63.9 ± 9.5	<0.001
Females			
Age (years)	30 ± 7	31 ± 5	0.51
Height (m)	1.6 ± 0.1	1.7 ± 0.1	<0.001
Weight (kg)	59.5 ± 10.0	61.4 ± 11.3	0.49
BMI (kg/m^2)	24.6 ± 4.8	22.0 ± 2.8	0.03
BSA (m^2)	1.6 ± 0.1	1.7 ± 0.2	0.06
Heart rate (per minute)	79.0 ± 15.1	64.8 ± 13.6	<0.001

[a]Values are shown as mean ± SD.
BMI = body mass index, BSA = body surface area.

dex normal ranges to the body surface area to allow for differences in body habitus, and estimate myocardial iron loading using the recently described T2* technique (12). We therefore used CMR to determine the "normal TM" range for LV volumes and function in subjects with a myocardial T2* of >20 msec, with additional appropriate normalization to body habitus. We hypothesized that the range for normality in chronically anemic TM patients would not agree with standard normal ranges, and that this would significantly impact the use of LV parameters to assess the presence of cardiomyopathy.

MATERIALS AND METHODS

Patients and Controls

The study population consisted of 205 patients (113 females and 92 males) with TM who were assessed for myocardial iron loading and ventricular function between 2001 and 2003. All of the patients had been regularly transfused since early childhood and had received chelation therapy since the mid to late 1970s or (for patients born after that time) from early childhood. A broad range of compliance with chelation therapy was reported. Only patients with normal myocardial T2* (>20 msec) were included in the study (12). Patients under the age of 18 were excluded because a substantial variability in ventricular volumes occurs during childhood and adolescence, and five patients were excluded because they had some other known cardiac pathology (pulmonary hypertension, aortic shelf, constrictive pericarditis, Fallot's tetralogy, or pulmonary artery stenosis). Since LV parameters differ with gender, we divided the population into male and female groups. Overall, the study population of 81 patients consisted of 37 males and 44 females. Normal control ranges were established from 40 age- and gender-matched healthy volunteers. The demographics of the patients and controls are shown in Table 1. Approval for these studies was received from the local ethics committee.

MRI

For myocardial T2* measurements we scanned the patients using a previously described multiecho technique (19). In brief, a single short-axis mid-ventricular slice was acquired at eight echo times (TE = 2.6–16.7 msec, 2-msec increments) in a single breath-hold, using even echoes to allow motion compensation. A gradient-echo sequence was used with a flip angle of 35°, a matrix of 128 × 256 pixels, a field of view (FOV) of 40 cm, and a sampling bandwidth of 810 Hz per pixel. The TR between the eight radiofrequency (RF) pulses applied each cardiac cycle was 20 msec. A delay time of 0 msec after the R-wave trigger was chosen to obtain a high-quality image when blood flow and myocardial wall motion artifacts were minimized. For analysis, a homogeneous full-thickness region of interest (ROI) was chosen in the ventricular septum that encompassed both epi- and endocardial regions. The signal intensity of this region was measured for each image and plotted against the TE to form an exponential decay curve using dedicated software (CMRtools; Cardiovascular Imaging Solutions, London, UK). To derive T2*, an exponential trend-line was fitted with an equation in the form $y = Ke^{-TE/T2^*}$, where K represents a constant, and y represents the image signal intensity.

LV volumes were acquired using standard techniques (20). In brief, breath-hold short-axis slices from the atrioventricular ring to apex were acquired with a 7-mm slice thickness and a 3-mm gap, one slice per breath-hold, using a true fast imaging with steady precession (TrueFISP) cine sequence. The number of cardiac phases varied between 14 and 18 (depending on the heart rate), and eight to 12 slices were required to cover the LV. Semiautomated software (CMRtools; Cardiovascular Imaging Solutions, London, UK) was used to derive the LV end-diastolic and end-systolic volumes, ejection fraction (EF), and mass. Since the body habitus may be below average in TM patients (21), all parameters were indexed to the body surface area, which was calculated from the patient's height and weight at the time of the assessment using the Mosteller formula (22). The average heart rate during the CMR assessment was noted. The cardiac output was calculated as the product of the heart rate and stroke volume.

Hemoglobin Concentration

Hemoglobin concentration estimations were performed in all patients just prior to the regular monthly transfusion of each patient. All MR scans were conducted within 1 week of the hemoglobin concentration estimation. The hemoglobin concentration estimation was based on the Advia 120 laser principle.

Statistical Analysis

Using the Kolmogorov-Smirnov test we found that the continuous data approximated to normal. Therefore, we calculated the means and standard deviations (SDs) for these parameters and compared them with those of the control group using an independent-sample *t*-test. Categorical data were compared by means of the Chi-squared test. Pearson's correlations were performed to assess the correlations of hemoglobin concentration

Table 2
LV Parameters Normalized to Body Surface Area for Males and Females With Mean and 95% Confidence Intervals, Divided Into TM Patients With No Myocardial Iron Loading and Nonanemic Age-Matched Controls*

	TM Patients Mean ± SD [95% CI]	Controls Mean ± SD [95% CI]	*P* value
Males			
LVEDVI (mL/m^2)	97.2 ± 27.2 [42-152]	84.1 ± 10.5 [63-105]	<0.05
LVESVI (mL/m^2)	23.1 ± 5.2 [13-34]	29.6 ± 6.1 [17-42]	<0.001
LVSVI (mL/m^2)	70.8 ± 15.8 [39-103]	54.4 ± 7.1 [40-69]	<0.001
LVEF	71.0 ± 6.1 [59-83]	64.9 ± 5.0 [55-75]	<0.001
LVMI (g/m^2)	84.6 ± 20.3 [44-125]	75.0 ± 8.4 [58-92]	<0.05
CO (L/minute)	9.8 ± 3.2 [3.4-16.2]	6.8 ± 1.5 [3.8-9.8]	<0.001
COI (L/minute/m^2)	5.7 ± 1.9 [1.9-9.5]	3.5 ± 0.7 [2.1-4.9]	<0.001
Females			
LVEDVI (mL/m^2)	87.4 ± 16.6 [54-121]	79.4 ± 9.8 [60-99]	<0.05
LVESVI (mL/m^2)	20.8 ± 7.3 [6-35]	26.1 ± 4.7 [17-36]	<0.01
LVSVI (mL/m^2)	66.5 ± 12.4 [42-91]	53.3 ± 7.3 [39-78]	<0.001
LVEF	75.1 ± 5.9 [63-87]	67.1 ± 4.3 [59-76]	<0.001
LVMI (g/m^2)	69.9 ± 17.3 [35-105]	61.9 ± 7.9 [46-78]	<0.05
CO (L/minute)	8.2 ± 2.0 [4.2 ± 12.2]	5.8 ± 1.6 [2.6-9.0]	<0.001
COI (L/minute/m^2)	5.2 ± 1.3 [2.6-7.8]	3.4 ± 0.8 [1.8-5.0]	<0.001

*The ejection fraction is not indexed to body surface area as it does not vary significantly with body habitus.
LVEDVI = left ventricular end-diastolic volume index, LVESVI = left ventricular end-systolic volume index, LVSVI = left ventricular stroke volume index, LVEF = left ventricular ejection fraction, LVMI = left ventricular mass index, CO = cardiac output, COI = cardiac output index.

with the derived LV volumes and function parameters. The data are shown as mean ± SD. A *P*-value < 0.05 was used for statistical significance.

RESULTS

Patient Characteristics

The TM patients were well matched to controls for gender and age (Table 1). However, the TM patients had significantly lower heights, weights, and body surface areas (both genders $P < 0.05$). In addition, the baseline heart rate was faster in TM patients (both genders $P < 0.001$). The body mass index (BMI), which adjusts weight for body surface area, was equivalent between TM patients and controls in males, and slightly increased in females.

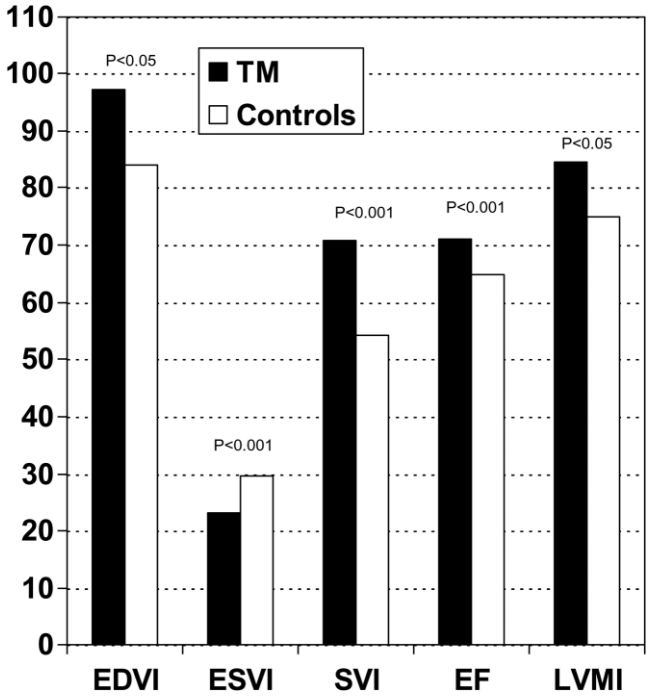

Figure 1. Comparison of LV volumes and function parameters between TM males and controls.

Ventricular Volumes and Function, and Cardiac Output

The results for ventricular volumes and function, and cardiac output are summarized in Table 2, and Figs. 1 and 2. In both males and females the TM cohort had a significantly increased LV end-diastolic volume ($P <$

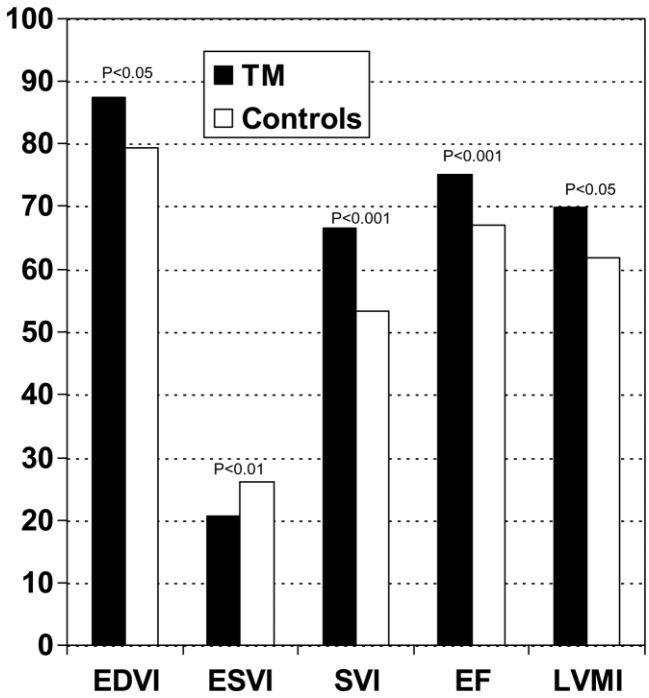

Figure 2. Comparison of LV volumes and function parameters between TM females and controls.

0.05), increased LVEF ($P < 0.001$), and increased LV mass ($P < 0.05$). The end-systolic volume was reduced ($P < 0.01$). The heart rates were higher in TM patients than in controls ($P < 0.001$). In combination with the increased stroke volume ($P < 0.001$) in the TM patients, this resulted in a significantly increased cardiac output ($P < 0.001$) and cardiac index ($P < 0.001$).

Correlation of Ventricular Parameters With Hemoglobin Concentration

The mean hemoglobin concentration was 10.7 ± 1.4g/dL in the females and 9.9 ± 1.5g/dL in the males. In the females there were no significant correlations between the hemoglobin concentration and any LV parameter. In the males there were inverse correlations between hemoglobin concentration and LV end-diastolic volume ($r = -0.47$, $P = 0.003$), LV stroke volume ($r = -0.41$, $P = 0.008$), cardiac output ($r = -0.52$, $P = 0.001$), and cardiac index ($r = -0.44$, $P = 0.006$).

DISCUSSION

Patients with TM anemia require regular, lifelong blood transfusions for survival. This leads to tissue iron accumulation, which is most significant in the heart. Heart failure resulting from cardiomyopathy is the reported cause of death in 71% of cases (23). A key clinical question, therefore, is how to detect and treat any cardiac involvement at an early stage. The recent development of T2* CMR allows rapid and reproducible assessments of myocardial iron levels (12,19), and it has been shown that cross-sectional analyses of markers of body iron status, such as serum ferritin levels and liver iron concentrations, have a poor correlation with myocardial T2* (12,13). Direct measures of LV function (usually obtained by echo) are more commonly used at present. However, such measures suffer from suboptimal reproducibility. A further limitation of such studies is that findings in TM patients are compared with normal ranges that are inappropriate. Patients with TM differ from healthy individuals in that they tend to be younger and are affected by the uncontrolled hemodynamic effects of anemia (i.e., the potential for increased heart size and cardiac output), a smaller body habitus due to growth retardation (i.e., decreased heart size and cardiac output), and the possible presence of cardiomyopathy (increased heart size and decreased cardiac function). Such a comparison is therefore flawed in principle.

In this study we greatly minimized the possibility of recruiting patients with occult iron overload cardiomyopathy by including only those patients with normal myocardial T2*. We also made appropriate adjustments for body habitus by indexing to the body surface area, a technique that is widely applied in cardiovascular medicine. The results show that all of the LV volume and function parameters in both males and females with TM differed significantly from those in the age- and gender-matched controls. In TM the end-diastolic volume is increased and the end-systolic volume is decreased, which leads to a large stroke volume and increased EF and cardiac output. The hyperdynamic circulation results in an increased LV mass. These findings in TM are consistent with previous studies in other chronic anemias (15).

In the females there were no significant correlations between ventricular function parameters and hemoglobin concentration. In the males there were significant inverse correlations between the serum hemoglobin concentration and end-diastolic volume, stroke volume, cardiac output, and cardiac index. This could be due to the slightly higher level of anemia present in the males, or it may be that some other factor affects females and males differently. However, in both the males and females there was no correlation of serum hemoglobin concentration with LVEF. This is similar to a recent observation by Davis et al (4) that there is no change in the LVEF pre- and post-transfusion irrespectively of the transfusion regimen given.

Previous studies reported that identifiable LV systolic dysfunction occurs late in the course of TM and carries a poor prognosis (24,25). However, these reports used reference ranges from non-anemic normals for comparison with TM patients. Since our study shows that "normal for TM" ranges (with a normal myocardial T2* and by inference minimal myocardial iron loading) are significantly different from ranges in healthy controls, this may explain in part why abnormalities of systolic function are seen late in the progression of iron-related cardiomyopathy. By way of example, the lower limit of "normal for TM" LVEF was significantly higher than the lower limit in controls (males 59 vs. 55%, females 63 vs. 59%, both $P < 0.001$). Therefore, cardiomyopathy may be present in TM patients even though their EF is still apparently in the lower normal range (a false-negative finding and reduced sensitivity for cardiomyopathy). However, opposite effects are seen with ventricular volumes. The upper limit of normal for the LV end-diastolic volume index was significantly higher in TM patients than controls (males 152 vs. 105 mL/m^2, females 121 vs. 99 mL/m^2; both $P < 0.05$), and therefore cardiomyopathy may not be present when the heart appears enlarged (false-positive finding and reduced specificity). It is important to use the "normal for TM" ranges for TM patients, since this may enhance diagnostic accuracy for detection of cardiomyopathy. Ultimately, the use of myocardial T2* to estimate myocardial iron directly may resolve this issue.

Limitations

It is known that tissue T2* reflects tissue iron; however, the calibration for myocardium is not yet known. This is an area of active research.

In conclusion, we found that LV volumes and systolic function in TM patients with normal myocardial T2* values were significantly different from those in healthy controls after we normalized for body habitus. Unless "normal for TM" reference ranges are used, diagnostic accuracy for cardiomyopathy will be significantly impaired, and in particular may lead to underestimation of systolic dysfunction.

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