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Myocardial iron overload in thalassaemia major. How early to check?

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Summary

The age at which it is necessary to start Cardiovascular Magnetic Resonance (CMR) T2* screening in thalassaemia major (TM) is still uncertain. To clarify this point, we evaluated the prevalence of myocardial iron overload (MIO), function and fibrosis by CMR in TM patients younger than 10 years. We retrospectively selected 35 TM patients enrolled in the Myocardial Iron Overload in Thalassaemia network. MIO was measured by T2* multislice multiecho technique. Biventricular function parameters were evaluated by cine images. To detect myocardial fibrosis, late gadolinium enhancement images were acquired. Patients' age ranged from 4·2 to 9·7 years. All scans were performed without sedation. Nine patients showed no MIO, 22 patients had heterogeneous MIO with a T2* global value ≥20 ms; two patients had heterogeneous MIO with a T2* global value <20 ms and two patients showed homogeneous MIO. No patient showed myocardial fibrosis. Among the patients with heart T2*<20 ms, the youngest was 6 years old, none showed heart dysfunction and the iron transfused was <35 g in all cases. Cardiac iron loading can occur much earlier than previously described. The first cardiac T2* assessment should be performed as early as feasible without sedation, especially if chelation is started late or if poor compliance is suspected.

Keywords: thalassaemia major, heart, magnetic resonance, iron overload, paediatric.

Cardiac disease secondary to iron overload remains the main cause of death in transfusion-dependent thalassaemia patients (Borgna-Pignatti *et al*, 2004). Cardiac magnetic resonance (CMR) provides a unique means to quantify cardiac iron loading non-invasively and with high reproducibilty (Anderson *et al*, 2001; Pepe *et al*, 2006a; Ramazzotti *et al*, 2009). In fact, the relaxation parameter T2* correlates inversely with myocardial iron overload (MIO) (Carpenter *et al*, 2011) and has been significantly associated with cardiac dysfunction (Anderson *et al*, 2001; Marsella *et al*, 2011).

A few studies have attempted to establish the age at which this parameter should be first measured in paediatric patients. In a study of 77 patients aged less than 18 years, Wood *et al* (2008) found cardiac iron to be present only in patients older than 9·5 years. All patients found to have cardiac iron had received at least 35 g of transfusional iron (Wood *et al*, 2008). Subsequently, a study from Brazil reported on 23 chronically transfused patients aged 7–18 years who had undergone magnetic resonance imaging (MRI). Cardiac iron was present in four of them, three of whom were males under the age of 10 years. All three had received irregular or late chelation therapy (Fernandes *et al*, 2009).

We retrospectively studied, by CMR, the prevalence of cardiac iron and function and myocardial fibrosis in a cohort of TM patients younger than 10 years, enrolled in a large cooperative study that included more than 2000 thalassaemia patients.

Materials and methods

Study population

The Myocardial Iron Overload in Thalassaemia (MIOT) network is an Italian cooperative group formed by eight MRI sites and 68 thalassaemia centres where MRI examinations are performed using homogeneous, standardized and validated procedures and where the patients' clinical and laboratory data are collected for scientific purposes in an

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electronically accessible centralized database (Meloni *et al*, 2009a; Ramazzotti *et al*, 2009). From the 2171 patients with haemoglobinopathies enrolled in the MIOT network, we retrospectively selected the 35 TM patients aged less than 10 years who had undergone at least one MRI scan.

All clinical and laboratory investigations were carried out at the thalassaemia centres where the patients were treated. The patients have been tested for endocrine co-morbidities according to the current criteria published for TM patients (De Sanctis *et al*, 2013; Pepe *et al*, 2013).

The study complied with the Declaration of Helsinki. For all patients, parents gave their informed consent. The project was approved by the institutional ethics committee.

Magnetic resonance imaging

MRI scans were performed using a 1.5 T scanner (GE Signa/Excite HD, Milwaukee, WI, USA). An eight-element cardiac phased-array receiver surface coil with breath-holding in end-expiration and electrocardiogram (ECG)-gating was used for signal reception.

The T2* technique was used for iron overload assessment. Its reproducibility and its transferability within the MIOT network had been previously demonstrated (Ramazzotti *et al*, 2009). For the heart, a multislice multiecho T2* approach was used. Three parallel short-axis views (basal, medium and apical) of the left ventricle (LV) were obtained. Each single short-axis view was acquired at nine echo times (TEs). Acquisition sequence details have been previously reported (Pepe *et al*, 2006a,b). For the liver, a single transverse slice was obtained at nine TEs using a T2* gradient–echo multiecho sequence (Positano *et al*, 2009). T2* images analysis was performed using a custom-written, previously validated software program (HIPPO MIOT®, Fondazione G. Monasterio CNR-Regione Toscana and Institute of Clinical Physiology, Pisa, Italy; Positano *et al*, 2007). The software provided the T2* value on each of 16 segments of the LV, according to the standard American Heart Association/American College of Cardiology (AHA/ACC) model (Cerqueira *et al*, 2002). The global heart T2* value was obtained by averaging all segmental T2* values and the T2* value in the mid-ventricular septum was obtained by averaging T2* values in the mid anterior septum and the mid inferior septum. A T2* measurement ≥20 ms was considered a 'conservative' normal value for all 16 segments and for the global heart T2* because it never falls below this threshold in normal subjects (Anderson *et al*, 2001; Positano *et al*, 2007). However, T2* calibration data suggest that 20 ms is equivalent to 1·1 mg/g iron dry weight (Carpenter *et al*, 2011), which is approximately twice the historically reported normal mean concentration of human myocardial iron (Collins & Taylor, 1987).

Cardiac iron concentration (CIC) was derived from T2* values using the formula described by Carpenter *et al* (2011). For the liver, the T2* value was calculated in a large region of interest (ROI) of standard dimension, chosen in a homogeneous area of parenchyma without blood vessels (Positano *et al*, 2009). Care was taken to avoid the ROI placement in the posterior lateral (VII) and medial (VIII) segments, which are more prone to susceptibility artifacts (Meloni *et al*, 2011a). A liver T2* <9·2 ms was considered indicative of a substantial load. Using the calibration curve introduced by Wood *et al* (2005), this cut-off corresponds to a liver iron concentration (LIC) higher than 3 mg/g dry weight (Angelucci *et al*, 2000).

For the quantification of biventricular function parameters, steady-state free procession cine images were acquired during 8-s breath holds in sequential 8-mm short-axis slices (gap 0 mm) from the atrio-ventricular ring to the apex. Images were analysed in a standard way using MASS® software (Medis, Leiden, The Netherlands). Except for ejection fraction (EF), indices of biventricular function parameters were calculated by the adjustment of body surface area. The inter-centre variability for the quantification of cardiac function had been previously reported (Marsella *et al*, 2011). The cut-offs used for the parameters of biventricular function were previously defined by us (Meloni *et al*, 2011b,c). In those studies, the investigated population included patients under the age of 18 years and did not include patients with myocardial fibrosis or an heterogeneous distribution of pathological T2* values, and images were analysed using the MASS® software. Heart dysfunction (HD) was diagnosed in presence of LV and/or right ventricular (RV) EF <2 standard deviations (SD) from the mean value normalized to age and gender.

To detect the presence of myocardial fibrosis, late gadolinium enhancement (LGE) images were acquired in a subset of patients, in the same view used for cine cardiac MRI from 10 to 18 min using a fast gradient-echo inversion recovery sequence. The LGE technique has been proved to be safe in thalassaemia patients (Meloni *et al*, 2009b). The contrast medium gadopentate dimeglumine (0·2 mmol/kg; Magnevist®, Bayer Schering Pharma, Berlin, Germany) was intravenously administered. Also, vertical, horizontal and oblique long-axis views were acquired. LGE was considered present whenever it was visualized in two different views (Pepe *et al*, 2009).

Results

Whole patient population

All MRI scans were performed without sedation. Demographic, clinical and MRI data of patients are summarized in Table I.

Thirty-three patients were Italian while two had recently arrived in Italy from South America. Patients' age ranged from 4·2 to 9·7 years. All patients were regularly transfused. At the time of the first MRI, three of them (8·6%) were not chelated. Of the 32 patients on chelation therapy at the time of CMR, 15 (46·9%) were using deferoxamine monotherapy (from 5 to

Table I. Demographic, clinical and MRI data of the 35 thalassaemia major patients aged less than 10 years.

Parameter	TM patients ($N = 35$)
Age (years)	7·7 ± 1·5
Sex (male/female)	22/13
Age at starting transfusions (years)	1·9 ± 1·3
Mean pre-transfusion Hb (g/l)	94 ± 9·0
Mean serum ferritin (μg/l)	2054 ± 1297
Patients on chelation therapy, *n* (%)	32 (91·4)
Age when chelation started (years)	3·4 ± 1·4
Global heart T2* (ms)	30·7 ± 7·7
Pathological segments (T2* < 20 ms), *n*	3·3 ± 4·1
MRI LIC (mg/g dry weight)	8·3 ± 7·1
LV EF (%)	63·0 ± 5·4
RV EF (%)	65·5 ± 5·9

MRI, Magnetic Resonance Imaging; LIC, liver iron concentration; LV, left ventricular; EF, ejection fraction; RV, right ventricular.

98 months, mean 37·0 ± 28·7 months), 10 (31·3%) were treated with deferasirox (from 1 to 43 months, mean 21·2 ± 15·2 months), 3 (9·4%) were using deferiprone alone (from 1 to 8 months, mean 4·4 ± 3·4 months), 3 (9·4%) were on combined therapy with deferoxamine and deferiprone (from 1 to 75 months, mean 29·1 ± 40·2 months) and 1 (3·1%) had been on sequential regimen with deferoxamine and deferiprone for the previous 9 months. Compliance was reported to be excellent in 16 patients (50%), good in 14 patients (43·8%), dubious in one patient (3·1%), and insufficient in only one patient (3·1%) treated with combined therapy with deferoxamine and deferiprone.

At the time of CMR no patient showed cardiac disease or endocrine co-morbidities.

The mean global heart T2* value was 30·7 ± 7·7 ms, corresponding to a mean MRI CIC of 0·78 ± 0·41 mg/g dry weight. Four patients (11·4%) showed a global heart T2* value <20 ms. Four groups of patients were identified by the segmental approach: nine patients (25·7%) showed no MIO (all 16 segmental T2* values ≥ 20 ms), 22 patients (62·9%) showed an heterogeneous MIO (some segments with T2* values ≥ 20 ms and other segments with T2* values < 20 ms) and a T2* global value ≥ 20 ms; two patients (5·7%) showed an heterogeneous MIO and a T2* global value < 20 ms and two patients (5·7%) had a homogeneous MIO (all segments with an abnormal T2* value; Fig 1).

Mean liver T2* was 6·7 ± 6·5 ms, corresponding to a mean MRI LIC of 8·3 ± 7·1 mg/g dry weight. Twenty-six patients (74·3%) had hepatic iron overload.

The global heart T2* was significantly correlated with the MRI LIC ($r = -0·448$; $P = 0·007$) and with the mean serum ferritin as measured for 12 months prior to the scan ($r = -0·333$, $P = 0·050$).

Biventricular function parameters were assessed only in 28/35 patients (80%), because a short MRI protocol was chosen in seven patients to avoid sedation. LV dysfunction (EF<54%) was found in one patient (male, 7 years old, treated with deferoxamine and showing an heterogeneous MIO with a global T2* value = 31·1 ms). No patient showed RV dysfunction.

Finally, 14 patients completed the MRI protocol with acquisition of the LGE images; none of them showed myocardial fibrosis.

Patients with significant myocardial iron overload

Table II reports the data of the four patients (three males and one female) with significant MIO (global heart T2* < 20 ms). All patients had a serum ferritin level > 1000 μg/l. Their serum ferritin levels were significantly higher than in the rest of the patients (3200 ± 968 μg/l vs. 1906 ± 1271 μg/l; $P = 0·043$).

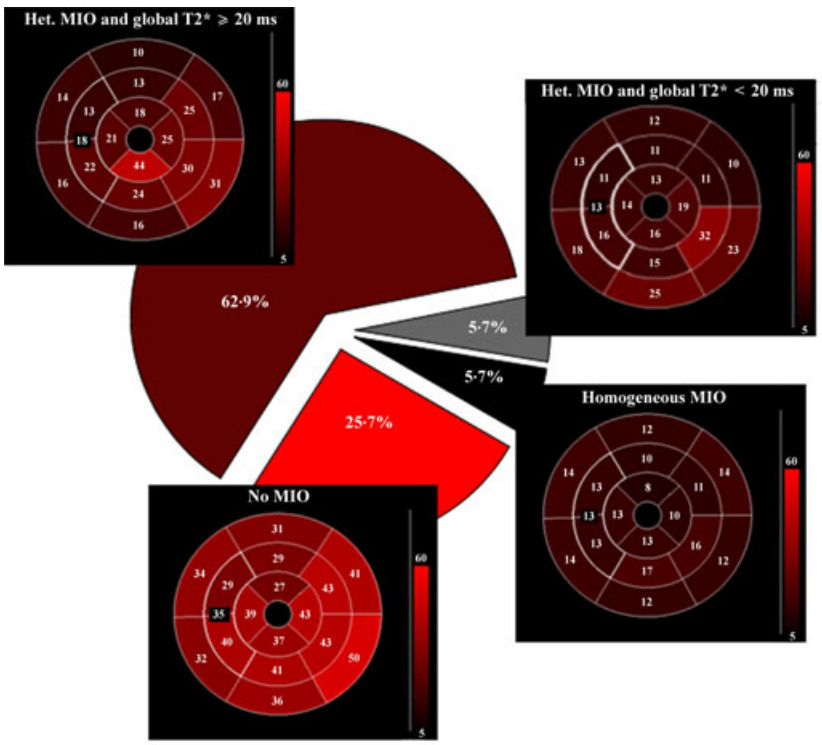
Hepatic iron overload was severe in three patients and moderate in one (Patient 3). Patient 2 had suffered from hepatitis related to cytomegalovirus. All four patients were chelated at the time of the first MRI. No patient had LV or RV dysfunction and one of them had been splenectomized.

After the first MRI, in order to reduce excess iron, Patient 1 was started on combination therapy with deferoxamine and deferiprone and the compliance was excellent. He underwent a follow-up MRI after 19 months and he again showed a homogeneous MIO. The global heart and the mid ventricular septum T2* values were both = 14 ms while the LIC was significantly decreased (from 21·4 to 7·5 mg/g dry weight). The liquid formulation of deferiprone was prescribed to Patient 2 and she showed a good compliance. She underwent a follow-up MRI after 12 months. The homogenous MIO became heterogeneous with global heart and mid-septum T2* values < 20 ms (18 and 16·5 ms, respectively). The LIC was 15·4 mg/g dry weight. Patient 3 continued deferasirox therapy but the dosage was increased to 35 mg/kg per day and a second MRI, performed after 18 months, revealed no MIO with a global heart T2* = 35 ms and a mid-septum T2* = 35·5 ms. No substantial hepatic ion overload was detected (LIC = 2·3 mg/g dry weight). After the first MRI, Patient 4 received a bone marrow transplantation and underwent iron removal by phlebotomy. A follow-up MRI after 28 months showed heterogeneous MIO with a global heart T2* value = 24 ms and a mid-ventricular septum = 28 ms. The LIC was 17·5 mg/g dry weight.

Discussion

Our data indicate that both cardiac and hepatic iron loading can occur much earlier than previously described and stress the importance of starting MRI evaluations of iron load as early as feasible.

Chelation therapy is of paramount importance in order to counteract the toxic effects of iron on the organs of multi-



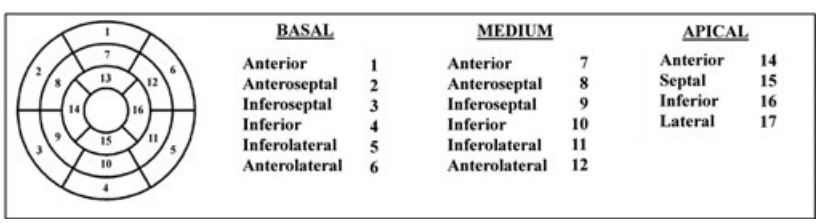


Fig 1. Up: Representative bull's eye maps identifying the four patterns of myocardial iron overload (MIO). The pie chart specifies the percentage of patients for each pattern. Bottom: Bull's-eye representation of the 16 myocardial standard segments.

transfused patients, particularly the heart and the liver (Maggio *et al*, 2009; Pepe *et al*, 2011). Iron balance is obtained when the daily excretion is sufficient to eliminate the iron introduced by transfusion, which, in most patients, ranges from 0·3 to 0·5 mg/kg per day. The age at which chelation should be started depends mainly on the amount of blood transfused. Most protocols recommend inception of therapy after transfusion of 10–15 units of blood or when ferritin exceeds 1000 μg/l (Anderson *et al*, 2001; Angelucci *et al*, 2008). MRI T2* can be used to monitor cardiac and liver iron burden non-invasively, reproducibly and accurately (Anderson *et al*, 2001; Pennell, 2008; Mavrogeni *et al*, 2011; Meloni *et al*, 2011a). Although insufficient compliance or high transfusion requirement could have played a role in patients' referral for MRI, many patients are now sent routinely from a very young age because of the increased availability of the technique.

The weak correlation between heart iron and LIC or serum ferritin confirms total body iron stores do not have strong immediate predictive value with respect to the presence of cardiac iron, even in paediatric patients younger than 10 years (Wood *et al*, 2008).

Previously published data suggest that iron accumulates in the heart late in the course of the disease and therefore it is often suggested that MRI needs not to be performed before 10 years of age when at least 35 g of iron have been transfused, although Wood *et al* (2008) proposed 8 years as the age to begin MRI surveillance. These conclusions were later challenged by a study showing that, out of 18 patients with thalassaemia major aged less than 18 years, two had a T2* <10 ms (Fernandes *et al*, 2009). MRI is an expensive procedure that is not available everywhere and, in addition, it is commonly held that young children are unable to undergo MRI without sedation. In our series of 35 patients younger than 10 years, selected retrospectively from a large data base of more than 2000 thalassaemia patients, sedation was never necessary and we were able to perform cardiac MRI without sedation as early as 5 years of age when the child was adequately prepared.

MRI demonstrated hepatic iron overload in 26 (74·3%) of the patients studied.

Based on a segmental heart T2* approach, a consistent number of patients (62·9%) showed an heterogeneous MIO but with a global heart T2* values > 20 ms (Fig 1). No prospective prognostic data are available about this subgroup of patients, who, given their early age, will need to be strictly followed up and monitored in terms of iron intake and che-

Table II. Demographic, clinical and MRI data of the four patients with global heart T2* <20 ms.

Parameter	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	9·5	6·8	8·8	7·9
Sex	Male	Female	Male	Male
Age at starting transfusions (months)	12	7	12	12
Mean Hb pre-transfusion (g/l)	90	97	98	96
Mean serum ferritin in the previous year (μg/l)	4500	2488	2579	2359
Transfused iron (g)	32	14	23	27
Age when chelation started (months)	16	30	24	36
Chelation treatment at the time of MRI	Deferoxamine	Deferasirox	Deferasirox	Deferoxamine
Compliance	Good	Dubious	Excellent	Excellent
Previous chelation therapy	None	Deferoxamine	Deferoxamine	Deferoxamine Deferasirox
Global heart T2*/Mid ventricular septum T2* (ms)	11·2/15	13/13	16·2/18	18·9/24·5
MRI CIC (mg/g dry weight)	2·35	1·97	1·51	1·25
Pathological segments (*n*)	16	16	12	9
Pattern of MIO	Homogenous	Homogenous	Heterogeneous	Heterogeneous
MRI LIC (mg/g dry weight)	21·4	23·3	9·6	15·1
LV EF (%)	61	NE	63	59
RV EF (%)	63	NE	64	56

MRI, Magnetic Resonance Imaging; CIC, cardiac iron concentration; MIO, myocardial iron overload; LIC, liver iron concentration; LV, left ventricular; EF, ejection fraction; RV, right ventricular; NE, not evaluated.

lation. A significant cardiac iron burden (global heart T2* <20 ms) was present in four patients (11%). The iron distribution was homogeneous in two and heterogeneous in the other two. Of the four patients with significant heart iron, three were younger than 9 years. Considering only the T2* of the mid ventricular septum, Patient 4 would have been incorrectly diagnosed as having no myocardial iron overload.

To date there is no information on the risks patients have of developing heart failure when heterogeneous deposition is present. Our preliminary data on a large cohort of TM adult population show that, compared to patients with no MIO, both patients with homogeneous MIO and patients with heterogeneous MIO and significant global heart iron were more likely to develop heart failure and heart dysfunction (Meloni *et al*, 2012). Moreover, prospectively, an homogeneous MIO identifies patients at high risk of heart failure (Meloni *et al*, 2013).

Although non-prospective data are available in paediatric population, a segmental T2* cardiac MR approach that could identify early iron deposits (Meloni *et al*, 2010), might therefore be useful for tailoring chelation therapy and preventing myocardial dysfunction in children. Although prospective data are not available for children, we suggest that the segmental approach could identify early the presence of cardiac iron, allowing intensification of therapy.

The EF was normal in all patients even when heart iron overload was significant. Severe iron overload in these patients was also shown by a liver T2* ranging from 1·1 to 2·7 ms (9·6–23·3 mg/g dry weight; Angelucci *et al*, 2000) and by serum ferritin levels that were significantly higher than the rest of the group ($P = 0·043$). The amount of iron transfused was <35 g in all four patients. This is in contrast with findings by Wood *et al* (2008), where there was no difference in ferritin or LIC between patients with and without detectable cardiac iron, although also in our study population we found a weak correlation between cardiac iron and total body iron stores.

All patients with significant MIO were receiving chelation therapy. Adherence was reported as good for three and dubious for one of the four patients, but it had not been quantitatively evaluated. Denial of poor compliance is a frequent event. Therapy was modified according to the MRI results, confirming the unique role of the MRI to tailor the chelation therapy in the iron-loaded patients.

In the Italian thalassaemia major population, myocardial fibrosis has been previously shown to be a relatively common finding (20%), correlating with age and hepatitis C virus (HCV) infection (Pepe *et al*, 2009), but it was not found in any of the thalassaemia major patients aged <10 years in the present study. Based on our data, the use of the contrast medium to detect myocardial fibrosis can be postponed until after 10 years of age.

We conclude that cardiac iron overload can appear earlier than previously believed. The first cardiac T2* assessment should be performed as early as it is possible without sedation and it is mandatory whenever poor compliance is suspected or if chelation has been started late. A more sensitive segmental approach to detected heart iron should be considered. The use of contrast medium can be delayed after 10 years age unless HCV infection or cardiac disease are present. Therapy can be modified on the basis of the MRI results and serious organ damage prevented.

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Author contributions

CBP and AP conceived the study and wrote the paper. AM performed the statistical analysis and was involved in preparing the manuscript. GG, AF, GBR, TC, and EC collected the data. LG was responsible for data collection. ML contributed to the interpretation of the results. All authors contributed to critical revision and final approval of the version to be published.

Conflicts of interest

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