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Cardiac complications and diabetes in thalassaemia major: a large historical multicentre study

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Summary

The relationship between diabetes mellitus (DM) and cardiac complications has never been systematically studied in thalassaemia major (TM). We evaluated a large retrospective historical cohort of TM to determine whether DM is associated with a higher risk of heart complications. We compared 86 TM patients affected by DM with 709 TM patients without DM consecutively included in the Myocardial Iron Overload in Thalassaemia database where clinical/instrumental data are recorded from birth to the first cardiovascular magnetic resonance (CMR) exam. All of the cardiac events considered were developed after the DM diagnosis. In DM patients *versus* non-DM patients we found a significantly higher frequency of cardiac complications (46·5% vs. 16·9%, $P < 0·0001$), heart failure (HF) (30·2% vs. 11·7%, $P < 0·0001$), hyperkinetic arrhythmias (18·6% vs. 5·5%, $P < 0·0001$) and myocardial fibrosis assessed by late gadolinium enhancement (29·9% vs. 18·4%, $P = 0·008$). TM patients with DM had a significantly higher risk of cardiac complications [odds ratio (OR) 2·84, $P < 0·0001$], HF (OR 2·32, $P = 0·003$), hyperkinetic arrhythmias (OR 2·21, $P = 0·023$) and myocardial fibrosis (OR 1·91, $P = 0·021$), also adjusting for the absence of myocardial iron overload assessed by T2* CMR and for the covariates (age and/or endocrine co-morbidity). In conclusion, DM significantly increases the risk for cardiac complications, HF, hyperkinetic arrhythmias and myocardial fibrosis in TM patients.

Keywords: cardiac complications, diabetes mellitus, thalassaemia major, myocardial iron overload, cardiovascular magnetic resonance.

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Thalassaemia major (TM) is a hereditary disease characterized by severe anaemia that requires regular transfusions, which can cause iron overload. The introduction of chelation therapy has improved the survival of these patients, but cardiomyopathies remain the main cause of mortality, while endocrinopathies are the most frequent morbidities. Diabetes mellitus (DM) is the third most common endocrine complication (Borgna-Pignatti *et al*, 2004). Different studies have reported a prominent role of iron overload, liver fibrosis and hepatitis C virus (HCV) infection in the development of the abnormal glucose metabolism, which it is characterized at an early stage by insulin resistance, followed by progressive insulin deficiency leading to overt DM (Gamberini *et al*, 2008; Mowla *et al*, 2004). The complex aetiopathogenesis of DM justifies why it is not used to differentiate type 1 or type 2 DM in TM (Gamberini *et al*, 2008; Mowla *et al*, 2004).

An association between DM and the risk of cardiovascular disease has been shown in the non-thalassaemic population (Butler *et al*, 1998). Diabetic cardiomyopathy has been recognized as a distinct clinical entity; it is characterized by metabolic abnormalities determining structural alterations (myocardial fibrosis and/or hypertrophy), functional changes, such as systolic and diastolic dysfunction, and clinical manifestations, such as heart failure (HF) and arrhythmias (Fang *et al*, 2004).

In TM patients myocardial iron overload (MIO) and chronic anaemia are the leading causes of cardiomyopathy, but other factors, such as myocarditis, pulmonary hypertension and endocrine abnormalities, play a role (De Sanctis *et al*, 2008; Pepe *et al*, 2009). Only one single-centre study on a small cohort of TM patients showed a significant higher prevalence of cardiac complications in diabetic *versus* non-diabetic patients (Gamberini *et al*, 2004). However, the relationship between DM and cardiac disease has never been studied in a systematic way in a large multi-centre TM population, using cardiac magnetic resonance (CMR) imaging.

This retrospective cohort study aimed to systematically evaluate in a large historical cohort of TM in the CMR era whether DM was associated with a higher risk of heart complications.

Methods

Study population

We considered data on 957 TM patients (48·7% males, mean age 30·8 ± 8·7 years), consecutively included in the Myocardial Iron Overload in Thalassaemia (MIOT) database where clinical and instrumental data are recorded from birth to the date of the first T2* CMR (September 2006-December 2010). Sixty-eight thalassaemia centres and 8 CMR sites are linked to the web-based MIOT database (Meloni *et al*, 2009a; Ramazzotti *et al*, 2009).

This study conforms with the principles outlined in the Declaration of Helsinki and was approved by the institutional ethics committees. All patients gave written informed consent.

Magnetic resonance imaging (MRI)

MRI examinations were performed at all sites with a 1·5T scanner (GE Signa/Excite HD, Milwaukee, WI, USA) using previously reported cardiac-gated techniques (Meloni *et al*, 2010; Pepe *et al*, 2006).

For the MIO measurements, a multislice multiecho T2* approach was used (Pepe *et al*, 2006). Three parallel short-axis views (basal, medium and apical) of the left ventricle (LV) were acquired. T2* image analysis was performed using custom-written, previously validated software (HIPPO MIOT®), able to map the myocardial T2* distribution into a 16-segment LV model according to the American Heart Association/American College of Cardiology (AHA/ACC) (Cerqueira *et al*, 2002). The global heart T2* value was obtained by averaging all segmental T2* values. The reproducibility of the methodology had been previously assessed (Pepe *et al*, 2006; Ramazzotti *et al*, 2009).

For quantification of the liver iron burden, the T2* value was calculated in a large region of interest of standard dimensions, chosen in a homogeneous area of parenchyma (Positano *et al*, 2009).

Steady-state free procession images were acquired in sequential 8-mm short-axis slices from the atrio-ventricular ring to the apex to assess biventricular function parameters quantitatively by standard methods, using MASS® software (Medis, Leiden, The Netherlands). The inter-centre variability had been previously reported (Marsella *et al*, 2011).

Late gadolinium enhanced (LGE) images were acquired 10–18 min after intravenous administration of Gadobutrol (1·0 mol/l, 0·2 mmol/kg) using a fast gradient-echo inversion recovery sequence. Short-axis vertical, horizontal, and oblique long-axis views were acquired. The LGE was evaluated visually by experienced observers and was considered present if visualized in two different views (Pepe *et al*, 2009). The transmural extent of LGE was defined as the extent of LGE >50% in each segment through the LV wall.

Diagnostic criteria

DM was defined by fasting plasma glucose ≥7·0 mmol/l or 2-h plasma glucose ≥ 11·1 mmol/l during an oral glucose tolerance test or a random plasma glucose ≥11·1 mmol/l (American Diabetes Association, 2011).

HF was identified by patient history, diagnosed by clinicians based on symptoms, signs and instrumental findings according to the AHA/ACC guidelines (Jessup *et al*, 2009). CMR was considered for the evaluation of the global systolic function and HF was identified when LV and/or right ventricular (RV) ejection fraction (EF) were ≤4 standard deviation (SD) from the mean value normalized to age and gender.

Heart dysfunction (HD) was diagnosed in presence of LVEF and/or RVEF <2 SD from the mean value normalized to age and gender. Previously defined cut-offs were followed for biventricular function parameters (Meloni *et al*, 2011).

Arrhythmias were diagnosed only if electrocardiogram-documented and requiring specific medication. Arrhythmias were classified according to the AHA/ACC Guidelines (Buxton *et al*, 2006).

Pulmonary hypertension was diagnosed if the trans-tricuspidal velocity jet was >3·2 m/s.

The term 'cardiac complications' included HF, arrhythmias and pulmonary hypertension that developed only after the DM diagnosis.

Non-MIO was defined when all segmental T2* values were greater or equal than the conservative normal cut-off of 20 ms. MIO was defined when at least one segment was lower than the conservative normal cut-off of 20 ms (Positano *et al*, 2007).

Statistical analysis

All data were analysed using SPSS version 13.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were described as mean ± SD and categorical variables as frequencies and percentages.

The normality of distribution of the parameters was assessed by using the Kolmogorov-Smirnov test. For continuous values, comparisons between groups were made by independent-samples *t*-test or by Wilcoxon's signed rank test. The chi-square test was used for non-continuous variables.

Odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic regression. ORs were adjusted for the covariates (age and/or endocrine co-morbidity) that were significantly different between groups and significantly associated with the dependent variable. An interaction term between DM and non-MIO was used to evaluate whether the effect of DM was different in MIO and non-MIO patients. In all tests, $P < 0{\cdot}05$ was considered to be statistically significant.

Results

Patient characteristics

Eighty-six (9%) out of 957 TM patients were affected by DM; 77 (88·4%) patients were under treatment with insulin, 7 (8·1%) with oral antiglycaemic agents, and 2 (3·5%) did not receive any therapy. The duration of DM was 13·9 ± 9·5 years.

Among the 871 patients without DM, 709 patients were selected according to the same age range as DM patients (19–51 years) and were considered as a comparison group.

Table I reports clinical data in patients with and without DM. Among MIO patients, no significant differences between DM and non-DM patients were found for global heart T2* (19·1 ± 10·2 vs. 20·9 ± 10·1 ms, $P = 0{\cdot}206$).

Cardiac end-points

In DM patients all cardiac complications occurred after the diagnosis of DM.

Table I. Comparison of demographic and clinical data in TM patients with and without DM.

	DM ($N = 86$)	No-DM ($N = 709$)	*P*-value
Sex (male/female)	35/51	350/359	0·129
Age (years)	37·4 ± 6·2	32·0 ± 6·7	<0·0001
Transfusions starting age (years)	1·2 ± 0·9	1·6 ± 1·6	0·325
Chelation starting age (years)	7·6 ± 6·5	4·9 ± 4·2	0·001
Splenectomy (%)	81·4	54·6	<0·0001
Mean pre-transfusion Hb (g/l) in the year before CMR	9·6 ± 0·6	9·6 ± 0·7	0·094
Serum Ferritin (μg/l)	1611 ± 2077	1379 ± 1279	0·492
DFO therapy at MRI (%)	36·1	35·9	0·933
Duration of DFO therapy at MRI (months)	160·1 ± 10·1	205·8 ± 158·6	0·236
Others chelation regimens (monotherapy, combined or sequential) with DFP (%)	49·4	43·5	0·365
Duration of other chelation regimens with DFP (months)	41·8 ± 56·4	46·1 ± 83·5	0·769
Current smoker (%)	15·4	16·3	0·837
Hypertension (%)	3·8	2·2	0·416
Chlolesterol (mmol/l)	3·27 ± 0·95	3·01 ± 0·78	0·083
Triglycerides (mmol/l)	1·36 ± 0·68	1·27 ± 0·66	0·524
Obesity (%)	5·2	4·8	0·782
Body Mass Index	22·2 ± 2·9	28·7 ± 52·8	0·252
HCV-antibody positive, (%)	89·5	80·6	0·044
HCV-RNA positive (%)	54·8	40	0·010
Endocrine co-morbidity (%)	76·7	49·2	<0·0001
Hypogonadism (%)	68·6	41·7	<0·0001
Hypothyroidism (%)	41·9	19·5	<0·0001
Hypoparathyroidism (%)	16·3	6·1	0·001
MIO (%)	66·3	53·6	0·026
MRI LIC (mg/g/dry weight)	7·8 ± 9·8	8·7 ± 11·5	0·205

DM, diabetes mellitus; CMR, cardiovascular magnetic resonance; DFO, desferrioxamine; MRI, magnetic resonance imaging; DFP, deferiprone; MIO, myocardial iron overload; LIC, liver iron concentration.

Cardiac complications were significantly higher in DM *versus* non-DM patients ($P < 0{\cdot}0001$) (Fig 1). In particular, a significant difference was found in the frequency of HF ($P < 0{\cdot}0001$) and hyperkinetic arrhythmias ($P < 0{\cdot}0001$) (Fig 1). The difference in the frequency of hyperkinetic arrhythmias was due to the supraventricular type (atrial tachycardia, atrial fibrillation and atrial flutter) (DM 16·3% *versus* non-DM 4·2%, $P < 0{\cdot}0001$). No differences were found in the frequency of hypokinetic arrhythmias (DM 0% *versus* non-DM 0·3%, $P = 1{\cdot}000$).

The frequency of pulmonary hypertension was comparable between DM (1·2%) and non-DM patients (2·1%); $P = 1{\cdot}000$.

Compared to patients without DM, patients with DM showed a significantly higher presence of myocardial fibrosis ($P = 0{\cdot}008$) (Fig 1). Among the patients with myocardial fibrosis, a coronary distribution with an ischemic transmural pattern was detected in 8·7% of DM patients *versus* 0·9% of non-DM patients ($P = 0{\cdot}084$). All patients positive for LGE with transmural pattern did not have a clinical history of previous myocardial infarction.

Heart dysfunction (LV and/or RV) was more frequent in DM (45·3%) than in non-DM patients (34·7%), but did not reach statistical significance ($P = 0{\cdot}052$). Between the two groups, the occurrence of RV dysfunction was significantly different (DM 18·6% vs. non-DM 11·1%, $P = 0{\cdot}044$), while no differences were found in the biventricular dysfunction ($P = 0{\cdot}162$) and LV dysfunction ($P = 0{\cdot}602$). No significant differences were found between DM and non-DM patients in the frequency of LV ($P = 0{\cdot}304$) and RV ($P = 0{\cdot}301$) dilatation, and in the left ($P = 0{\cdot}507$) and right ($P = 0{\cdot}357$) atrial areas.

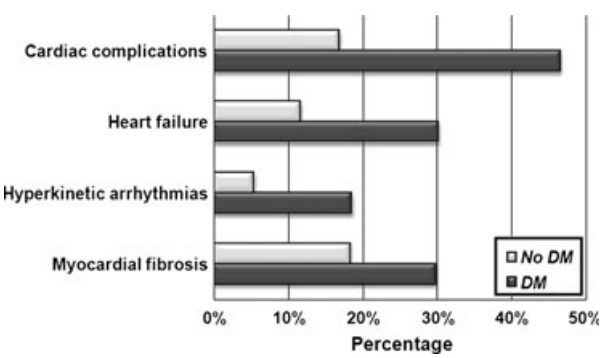

Fig 1. Prevalence of significant cardiac end-points for patients with and without diabetes mellitus (DM).

Relationship between DM and cardiac involvement

No significant differences in the DM duration were found between the patients with no MIO and the patients with MIO (16·9 ± 10·0 vs. 13·3 ± 9·0; $P = 0{\cdot}077$).

To evaluate the impact of the DM on cardiac involvement also regarding the absence of cardiac iron burden, we always adjusted the risk of cardiac findings for non-MIO (Table II).

DM patients were significantly more likely to have overall cardiac complications, HF, hyperkinetic arrhythmias and myocardial fibrosis, also adjusting for age and endocrine co-morbidity (Fig 2).

No significant interaction between DM and non-MIO was found for cardiac complications ($P = 0{\cdot}197$), HF ($P = 0{\cdot}686$), myocardial fibrosis ($P = 0{\cdot}645$) and heart dysfunction ($P = 0{\cdot}299$). Thus, the DM effect was comparable in MIO and non-MIO patients.

Given that the interaction between DM and heart iron was significant for hyperkinetic arrhythmias ($P = 0{\cdot}016$), we analysed the transverse risk for hyperkinetic arrhythmias correlated to DM in non-MIO and MIO patients. In patients without heart iron the risk for hyperkinetic arrhythmias was significantly higher for DM, also adjusting for age and endocrine co-morbidity (OR 3·87, CI 1·43–10·49; $P = 0{\cdot}008$). Conversely, in patients with heart iron the risk for hyperkinetic arrhythmias was not significantly higher for DM (OR 1·91, CI 0·74–4·49; $P = 0{\cdot}180$). The risk for hyperkinetic arrhythmias, adjusted for age and endocrine co-morbidity, was significantly lower in DM patients with MIO (OR 0·19, CI 0·05–0·69; $P = 0{\cdot}012$), and was similar in non-DM

Table II. Logistic regression analysis: Odds Ratios and 95% confidence intervals of DM *versus* non-DM patients for cardiac findings.

	Adjusted for non-MIO		Adjusted for non-MIO and covariates	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Cardiac complications	4·23 (2·65–6·76)	<0·0001	2·84 (1·71–4·69)‡	<0·0001
Heart failure	3·14 (1·87–5·26)	<0·0001	2·33 (1·33–4·06)‡	0·003
Hyperkinetic arrhythmias	4·09 (2·16–7·74)	<0·0001	2·21 (1·12–4·37)‡	0·023
Myocardial fibrosis	2·12 (1·24–3·63)	0·006	1·91 (1·11–3·29)†	0·021
Heart dysfunction (LV and/or RV)	1·45 (0·37–2·33)	0·093		
Biventricular dysfunction	1·46 (0·78–2·72)	0·235		
LV dysfunction	0·77 (0·37–1·60)	0·487	0·93 (0·44–1·98)*	0·855
RV dysfunction	1·82 (1·01–3·30)	0·048	1·33 (0·71–2·49)*	0·366

Covariates: *age; †Endocrine co-morbidity; ‡Age and endocrine co-morbidity.

OR, odds ratio; 95% CI, 95% confidence interval; DM, diabetes mellitus; MIO, myocardial iron overload; LV, left ventricle; RV, right ventricle.

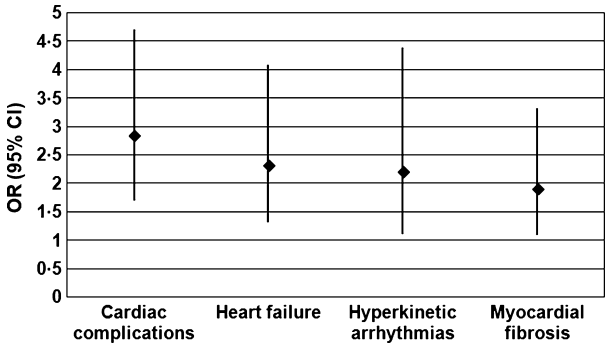

Fig 2. Logistic regression analysis: ORs (95% CI) of DM *versus* non-DM patients for significant cardiac end-points. DM, diabetes mellitus; OR, odds ratio; 95% CI, 95% confidence interval.

patients with or without MIO (OR 1·22, CI 0·61–2·44; $P = 0·572$).

In our study population the risk for heart dysfunction was significantly higher in MIO *versus* non-MIO patients (OR 1·65, CI 1·23–2·22; $P = 0·001$).

No independent effect of HCV-RNA was observed for any cardiac endpoint. A near-significant interaction between DM and HCV-RNA was found for myocardial fibrosis ($P = 0·056$). Adjusting for MIO and for other endocrine co-morbidity in comparison with the non-DM and non-HCV-RNA patients, the risk for myocardial fibrosis was not significantly higher in patients with only HCV-RNA positivity (OR 0·98, CI 0·63–1·55; $P = 0·946$) or in patients with only DM (OR 0·86, CI 0·34–2·20; $P = 0·864$), but the risk was significantly higher in patients who were positive for both DM and HCV-RNA (OR 2·69, CI 1·31–5·55; $P = 0·007$).

The DM duration was not significantly different between the patients with or without cardiac complications ($11·1 \pm 9·1$ vs. $14·3 \pm 9·6$; $P = 0·135$), patients with or without heart failure ($12·8 \pm 9·3$ vs. $13·9 \pm 9·7$; $P = 0·671$) and patients with or without myocardial fibrosis ($16·4 \pm 8·8$ vs. $13·3 \pm 9·8$; $P = 0·175$). Conversely, the duration of the DM was significantly shorter in patients with hyperkinetic arrhythmias than in patients without hyperkinetic arrhythmias ($7·83 \pm 7·01$ vs. $15·31 \pm 9·26$; $P = 0·006$).

Discussion

In rare diseases such as thalassaemia, collaborative projects like the MIOT network are recommended to produce evidence that aids better management of patients. We retrospectively assessed the relationship between cardiac complications and DM in a large historical cohort of TM patients, homogenous for optimized transfusion and chelation treatments. Moreover, all patients underwent CMR examination assessing heart iron burden, bi-ventricular function parameters and myocardial fibrosis with a high level of reproducibility (Marsella *et al*, 2011; Pepe *et al*, 2009; Ramazzotti *et al*, 2009).

In our TM population, the frequency of DM was 9%, higher than the value of 5·4% previously reported in a study population that was comparable in size, but younger (Italian Working Group on Endocrine Complications in Non-endocrine Diseases, 1995). The increased prevalence is probably the consequence of the increased longevity of the thalassaemia population. In fact, a longitudinal study performed in a single Italian centre showed that the overall prevalence of DM increased progressively over the years (Gamberini *et al*, 2008).

The DM patients were significantly older, started chelation therapy significantly later and showed higher cardiac iron burden and significantly higher frequency of HCV hepatitis. These data confirm the prominent role of iron overload, liver fibrosis and HCV infection reported in the development of the abnormal glucose metabolism in thalassaemia patients (Gamberini *et al*, 2008; Mowla *et al*, 2004).

No significant differences were found between the patients with and without DM with regard to desferrioxamine (DFO) therapy or to other chelation regimens with deferiprone (DFP) (in monotheraphy, combined or sequential) and in their durations at the time of CMR (Table I).

The high endocrine co-morbidity observed in TM patients with DM is in agreement with data reported in the literature (Gamberini *et al*, 2004). The siderosis may explain this multi-organ endocrine failure, as indirectly supported by the higher level of heart iron and as shown in a previous cross-sectional and longitudinal study (Gamberini *et al*, 2008).

Relationship between DM and cardiac involvement

TM patients with DM showed an increased frequency of cardiac complications (heart failure, arrhythmias and pulmonary hypertension) (Fig 1). The influence of DM on cardiac complications was comparable in patients with or without MIO and the risk for cardiac complications was significantly higher also adjusting for age and endocrine co-morbidity (Fig 2). The influence of previous and different chelation regimens could explain why no significant difference was found in the DM duration between the patients with no MIO and MIO.

In our cohort we confirmed a low frequency of pulmonary hypertension in well-treated TM forms (Derchi *et al*, 1999; Pepe *et al*, 2009) and DM did not influence the frequency of pulmonary hypertension.

Our data further upheld heart failure as the main cardiac complication in TM patients, even when well-treated and well-chelated (Borgna-Pignatti *et al*, 2004). TM patients with DM showed increased frequency of HF (Fig 1) with a significantly higher risk, also when adjusting for age and endocrine co-morbidity (Fig 2). The influence of DM on HF was comparable in patients with or without MIO. Our data are concordant with the robust evidence regarding the relationship between DM and HF in the general population (Maisch *et al*, 2011).

In our large well-treated population, hyperkinetic arrhythmias (mainly represented by supraventricular forms) were

the second most frequent cardiac complication, as previously reported (Borgna-Pignatti *et al*, 2004). In patients without MIO the risk of hyperkinetic arrhythmias was significantly higher for DM patients, also adjusting for age and endocrine co-morbidity, suggesting an independent role of diabetic cardiomyopathy in hyperkinetic arrhythmias in thalassaemia. Moreover, in DM patients without MIO the risk for hyperkinetic arrhythmias, when adjusted for age and endocrine co-morbidity, was significantly higher than in DM patients with MIO. These findings confirm that, as previously reported (Kirk *et al*, 2009; Marsella *et al*, 2011), cardiac iron overload contributes less to the development of arrhythmias than to cardiac failure. DM seems to increase the stiffness of the ventricle independently of MIO. In contrast, iron-overloaded hearts were probably so stiff already that the additional diagnosis of DM did not make much of a difference. Also, in the general population DM patients show a higher incidence of cardiac arrhythmias, although the physiological basis is not completely known (Schannwell *et al*, 2002). However, the higher incidence of hyperkinetic arrhythmias in DM was expected, due to the greater stiffness of the diabetic ventricles where microvascular damage probably plays a key role (Schannwell *et al*, 2002).

Our study strongly supports that DM is positively associated with myocardial fibrosis in TM, as this was the fibrosis detected by LGE. LGE has been used to document the presence of myocardial fibrosis in the general DM population (Kwong *et al*, 2008; Maisch *et al*, 2011) and myocardial fibrosis by LGE has been previously shown in TM patients (Kirk *et al*, 2011; Meloni *et al*, 2009b,c; Pepe *et al*, 2009). The frequency of myocardial fibrosis in this large Italian cohort of TM patients (Meloni *et al*, 2009b,c; Pepe *et al*, 2009) was significantly higher than that detected in a small study group of English TM patients (Kirk *et al*, 2011) (157/833 patients *versus* 1/45 patients; $P = 0{\cdot}004$). The significantly higher frequency of HCV infection in the Italian TM population might resolve these differences in LGE frequency. As shown in this study, the risk for myocardial fibrosis was significantly higher in patients that were positive for both DM and HCV-RNA. Thus, HCV infection seems to be one of the possible mechanisms for myocardial fibrosis both directly through myocarditis (Omura *et al*, 2005) and indirectly through pancreas and liver damage with the development of diabetes.

DM appears to reinforce the oxidative stress and cardiac microvascular angiopathy produced by HCV infection. Moreover, in DM patients the higher frequency of a positive LGE with a transmural ischaemic pattern also confirms the higher risk for coronary artery disease in diabetic TM and the role of the macroangiopathy in diabetic cardiomyopathy.

The frequency of heart dysfunction was higher in DM patients, with a *P*-value (0·052) close to statistical significance. The lack of association between DM and heart dysfunction probably reflects the predominant role of the iron burden in mild heart systolic dysfunction in thalassaemia; moreover, this finding might be due to the small number of cases with mild heart systolic dysfunction alone. The comparable data regarding LV/RV dilatation and the atrial areas reflect the predominant role of chronic anaemia *versus* diabetic cardiomiopathy in conditioning the remodelling of the four cardiac chambers.

The lack of correlation between DM duration and cardiac complications probably reflects two biases. First of all, there is a potential delay between the DM diagnosis and the real onset of the disease; this delay could fluctuate due to the different standard care in the various centres, particularly in the past. Second, although the physiological basis for arrhythmias in diabetic cardiomyopathy is not completely known, the greater stiffness of diabetic ventricles probably plays a key role (Schannwell *et al*, 2002) and the onset of cardiac active therapy (i.e. ACE-I), usually years after DM diagnosis, could have prevented arrhythmias in patients where the DM duration was longer.

In our retrospective historical cohort, the hypothesis that the presence of DM leads to higher frequency of cardiac complications also independently of cardiac iron status appears to be fully confirmed. In fact, DM damages the cardiac vessels, especially the microvasculature. Microvascular disease has long been felt to be predominantly present in the eyes, kidneys and nerves. However, it is becoming increasingly clear that this process also affects the microvasculature of the heart (Butler *et al*, 1998; Fang *et al*, 2004).

Limitations

An interaction term between DM and non-MIO was used to evaluate whether the effect of DM was different in MIO and non-MIO patients. Unfortunately, the evaluation for MIO was performed only at the time of the first T2* CMR examination because non-invasive techniques for MIO quantification were previously unavailable.

Moreover, our retrospective cohort did not have data regarding pancreatic iron burden because the T2* pancreatic technique was not implemented for clinical use in the MIOT network until 2012 (Restaino *et al*, 2011), when we also introduced the systematic collection of data (not available in this study) regarding impaired glucose tolerance and impaired fasting glucose in our patients.

Cardiac iron is more specific for glucose dysregulation because it implies increased pancreatic iron for a long duration (Noetzli *et al*, 2009), but it is insensitive to milder glucose. While pancreas T2* has greater variability than liver or cardiac T2* (Restaino *et al*, 2011), the disposition index (a balance between insulin sensitivity and insulin release) could be an important marker of intrinsic beta cell functional capacity and diabetes reversibility (Noetzli *et al*, 2012).

Another limit of this study is that the resolution of LGE imaging is insufficient to visualize diffuse myocardial fibrosis resulting from microvascular disease in diabetic cardiomyopathy, though the resolution of CMR for this purpose is

significantly greater than any other modality. One possible way to assess this issue in future patient settings would be the use of CMR T1 mapping techniques for the LV myocardium.

Conclusions

Cardiovascular complications remain the leading cause of mortality and morbidity in the thalassaemia population as well as in the general diabetes population. Our data, from a large historical cohort of TM patients, appear to indicate that DM increases independently the risk for heart failure, hyperkinetic arrhythmias and myocardial fibrosis in TM. Our data are concordant with recent research in humans and animals that revealed pathophysiological mechanisms for the increased vulnerability of the diabetic heart to failure, and arrhythmias in the general population(Fang *et al*, 2004; Hess *et al*, 2012), although experimental findings and prospective data are recommended to further support the association between DM and cardiac complications in thalassaemia. However, our data are relevant for the prevention of glucose disorder metabolism, particularly in young patients, and they stress the need to intensify the chelation therapy in patients in whom excess pancreatic iron is found by MRI where available, or when patients develop glucose metabolism disorders, because, as previously shown (Farmaki *et al*, 2010), improvement is possible.

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Authorship contributions

AP and MRG conceived the study and wrote the paper. ML conceived the study. AM and GR performed the statistical analysis. VC, LC, AS, CG, AZ, DGD, SG, MS, SC, MEL, LP, EC, CA collected the data. LG was responsible for data collection. VP was responsible for data analysis. All authors contributed to critical revision and final approval of the version to be published.

Conflicts of interest

The authors do not have any conflict of interest to declare.

References

American Diabetes Association (ADA) (2011) Diagnosis and classification of diabetes mellitus. *Diabetes Care*, **34**, S62–S69.

Borgna-Pignatti, C., Rugolotto, S., De Stefano, P., Zhao, H., Cappellini, M.D., Del Vecchio, G.C., Romeo, M.A., Forni, G.L., Gamberini, M.R., Ghilardi, R., Piga, A. & Cnaan, A. (2004) Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica*, **89**, 1187–1193.

Butler, R., MacDonald, T.M., Struthers, A.D. & Morris, A.D. (1998) The clinical implications of diabetic heart disease. *European Heart Journal*, **19**, 1617–1627.

Buxton, A.E., Calkins, H., Callans, D.J., DiMarco, J.P., Fisher, J.D., Greene, H.L., Haines, D.E., Hayes, D.L., Heidenreich, P.A., Miller, J.M., Poppas, A., Prystowsky, E.N., Schoenfeld, M.H., Zimetbaum, P.J., Goff, D.C., Grover, F.L., Malenka, D.J., Peterson, E.D., Radford, M.J. & Redberg, R.F. (2006) ACC/AHA/HRS 2006 key data elements and definitions for electrophysiological studies and procedures: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Data Standards (ACC/AHA/HRS Writing Committee to Develop Data Standards on Electrophysiology). *Circulation*, **114**, 2534–2570.

Cerqueira, M.D., Weissman, N.J., Dilsizian, V., Jacobs, A.K., Kaul, S., Laskey, W.K., Pennell, D.J., Rumberger, J.A., Ryan, T. & Verani, M.S. (2002) Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart: a statement for healthcare professionals

from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. *Circulation*, **105**, 539–542.

De Sanctis, V., Govoni, M.R., Sprocati, M., Marsella, M. & Conti, E. (2008) Cardiomyopathy and pericardial effusion in a 7 year-old boy with beta-thalassaemia major, severe primary hypothyroidism and hypoparathyroidism due to iron overload. *Pediatric Endocrinology Reviews*, **6**, 181–184.

Derchi, G., Fonti, A., Forni, G.L., Galliera, E.O., Cappellini, M.D., Turati, F. & Policlinico, O.M. (1999) Pulmonary hypertension in patients with thalassemia major. *American Heart Journal*, **138**, 384.

Fang, Z.Y., Prins, J.B. & Marwick, T.H. (2004) Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications. *Endocrine Reviews*, **25**, 543–567.

Farmaki, K., Tzoumari, I., Pappa, C., Chouliaras, G. & Berdoukas, V. (2010) New golden era of chelation therapy in thalassaemia: the achievement and maintenance of normal range body iron stores. Response to Kontoghiorghes et al. *British Journal of Haematology*, **150**, 491.

Gamberini, M.R., Fortini, M., De Sanctis, V., Gilli, G. & Testa, M.R. (2004) Diabetes mellitus and impaired glucose tolerance in thalassaemia major: incidence, prevalence, risk factors and survival in patients followed in the Ferrara Center. *Pediatric Endocrinology Reviews*, **2**, 285–291.

Gamberini, M.R., De Sanctis, V. & Gilli, G. (2008) Hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism: incidence and prevalence related to iron overload and chelation therapy in patients with thalassaemia major followed from 1980 to 2007 in the Ferrara Centre. *Pediatric Endocrinology Reviews*, **6**, 158–169.

Hess, K., Marx, N. & Lehrke, M. (2012) Cardiovascular disease and diabetes: the vulnerable patient. *European Heart Journal Supplements*, **14**, B4–B13.

Italian Working Group on Endocrine Complications in Non-endocrine Diseases (1995) Multicentre study on prevalence of endocrine complications in thalassaemia major. *Clinical Endocrinology (Oxf)*, **42**, 581–586.

Jessup, M., Abraham, W.T., Casey, D.E., Feldman, A.M., Francis, G.S., Ganiats, T.G., Konstam, M.A., Mancini, D.M., Rahko, P.S., Silver, M.A., Stevenson, L.W. & Yancy, C.W. (2009) 2009 focused update: ACCF/AHA Guidelines for the Diagnosis and Management of Heart Failure in Adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. *Circulation*, **119**, 1977–2016.

Kirk, P., Roughton, M., Porter, J.B., Walker, J.M., Tanner, M.A., Patel, J., Wu, D., Taylor, J., Westwood, M.A., Anderson, L.J. & Pennell, D.J. (2009) Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. *Circulation*, **120**, 1961–1968.

Kirk, P., Carpenter, J.P., Tanner, M.A. & Pennell, D.J. (2011) Low prevalence of fibrosis in thalassemia major assessed by late gadolinium enhancement cardiovascular magnetic resonance. *Journal of Cardiovascular Magnetic Resonance*, **13**, 8.

Kwong, R.Y., Sattar, H., Wu, H., Vorobiof, G., Gandla, V., Steel, K., Siu, S. & Brown, K.A. (2008) Incidence and prognostic implication of unrecognized myocardial scar characterized by cardiac magnetic resonance in diabetic patients without clinical evidence of myocardial infarction. *Circulation*, **118**, 1011–1020.

Maisch, B., Alter, P. & Pankuweit, S. (2011) Diabetic cardiomyopathy–fact or fiction? *Herz*, **36**, 102–115.

Marsella, M., Borgna-Pignatti, C., Meloni, A., Caldarelli, V., Dell'Amico, M.C., Spasiano, A., Pitrolo, L., Cracolici, E., Valeri, G., Positano, V., Lombardi, M. & Pepe, A. (2011) Cardiac iron and cardiac disease in males and females with transfusion-dependent thalassemia major: a T2* magnetic resonance imaging study. *Haematologica*, **96**, 515–520.

Meloni, A., Ramazzotti, A., Positano, V., Salvatori, C., Mangione, M., Marcheschi, P., Favilli, B., De Marchi, D., Prato, S., Pepe, A., Sallustio, G., Centra, M., Santarelli, M.F., Lombardi, M. & Landini, L. (2009a) Evaluation of a web-based network for reproducible T2* MRI assessment of iron overload in thalassemia. *International Journal of Medical Informatics*, **78**, 503–512.

Meloni, A., Favilli, B., Positano, V., Cianciulli, P., Filosa, A., Quarta, A., D'Ascola, D., Restaino, G., Lombardi, M. & Pepe, A. (2009b) Safety of cardiovascular magnetic resonance gadolinium chelates contrast agents in patients with hemoglobinopaties. *Haematologica*, **94**, 1625–1627.

Meloni, A., Pepe, A., Positano, V., Favilli, B., Maggio, A., Capra, M., Lo Pinto, C., Gerardi, C., Santarelli, M.F., Midiri, M., Landini, L. & Lombardi, M. (2009c) Influence of myocardial fibrosis and blood oxygenation on heart T2* values in thalassemia patients. *Journal of Magnetic Resonance Imaging*, **29**, 832–837.

Meloni, A., Positano, V., Pepe, A., Rossi, G., Dell'Amico, M., Salvatori, C., Keilberg, P., Filosa, A., Sallustio, G., Midiri, M., D'Ascola, D., Santarelli, M.F. & Lombardi, M. (2010) Preferential patterns of myocardial iron overload by multislice multiecho T*2 CMR in thalassemia major patients. *Magnetic Resonance in Medicine*, **64**, 211–219.

Meloni, A., Dell'Amico, M., Favilli, B., Aquaro, G., Festa, P., Chiodi, E., Renne, S., Galati, M., Sardella, L., Keilberg, P., Positano, V., Lombardi, M. & Pepe, A. (2011) Left Ventricular Volumes, Mass and Function normalized to the body surface area, age and gender from CMR in a large cohort of well-treated Thalassemia Major patients without myocardial iron overload. *Journal of Cardiovascular Magnetic Resonance*, **13**, P305.

Mowla, A., Karimi, M., Afrasiabi, A. & De Sanctis, V. (2004) Prevalence of diabetes mellitus and impaired glucose tolerance in beta-thalassemia patients with and without hepatitis C virus infection. *Pediatric Endocrinology Reviews*, **2**, 282–284.

Noetzli, L.J., Papudesi, J., Coates, T.D. & Wood, J.C. (2009) Pancreatic iron loading predicts cardiac iron loading in thalassemia major. *Blood*, **114**, 4021–4026.

Noetzli, L.J., Mittelman, S.D., Watanabe, R.M., Coates, T.D. & Wood, J.C. (2012) Pancreatic iron and glucose dysregulation in thalassemia major. *American Journal of Hematology*, **87**, 155–160.

Omura, T., Yoshiyama, M., Hayashi, T., Nishiguchi, S., Kaito, M., Horiike, S., Fukuda, K., Inamoto, S., Kitaura, Y., Nakamura, Y., Teragaki, M., Tokuhisa, T., Iwao, H., Takeuchi, K. & Yoshikawa, J. (2005) Core protein of hepatitis C virus induces cardiomyopathy. *Circulation Research*, **96**, 148–150.

Pepe, A., Positano, V., Santarelli, F., Sorrentino, F., Cracolici, E., De Marchi, D., Maggio, A., Midiri, M., Landini, L. & Lombardi, M. (2006) Multislice multiecho T2* cardiovascular magnetic resonance for detection of the heterogeneous distribution of myocardial iron overload. *Journal of Magnetic Resonance Imaging*, **23**, 662–668.

Pepe, A., Positano, V., Capra, M., Maggio, A., Lo Pinto, C., Spasiano, A., Forni, G., Derchi, G., Favilli, B., Rossi, G., Cracolici, E., Midiri, M. & Lombardi, M. (2009) Prevalence and clinical-Instrumental correlates of myocardial scarring by delayed enhancement cardiovascular magnetic resonance in thalassemia major. *Heart*, **95**, 1688–1693.

Positano, V., Pepe, A., Santarelli, M.F., Scattini, B., De Marchi, D., Ramazzotti, A., Forni, G., Borgna-Pignatti, C., Lai, M.E., Midiri, M., Maggio, A., Lombardi, M. & Landini, L. (2007) Standardized T2* map of normal human heart in vivo to correct T2* segmental artefacts. *NMR in Biomedicine*, **20**, 578–590.

Positano, V., Salani, B., Pepe, A., Santarelli, M.F., De Marchi, D., Ramazzotti, A., Favilli, B., Cracolici, E., Midiri, M., Cianciulli, P., Lombardi, M. & Landini, L. (2009) Improved T2* assessment in liver iron overload by magnetic resonance imaging. *Magnetic Resonance Imaging*, **27**, 188–197.

Ramazzotti, A., Pepe, A., Positano, V., Rossi, G., De Marchi, D., Brizi, M.G., Luciani, A., Midiri, M., Sallustio, G., Valeri, G., Caruso, V., Centra, M., Cianciulli, P., De Sanctis, V., Maggio, A. & Lombardi, M. (2009) Multicenter validation of the magnetic resonance t2* technique for segmental and global quantification of myocardial iron. *Journal of Magnetic Resonance Imaging*, **30**, 62–68.

Restaino, G., Meloni, A., Positano, V., Missere, M., Rossi, G., Calandriello, L., Keilberg, P., Mattioni, O., Maggio, A., Lombardi, M., Sallustio, G. & Pepe, A. (2011) Regional and global pancreatic T*(2) MRI for iron overload assessment in a large cohort of healthy subjects: normal values and correlation with age and gender. *Magnetic Resonance in Medicine*, **65**, 764–769.

Schannwell, C.M., Schneppenheim, M., Perings, S., Plehn, G. & Strauer, B.E. (2002) Left ventricular diastolic dysfunction as an early manifestation of diabetic cardiomyopathy. *Cardiology*, **98**, 33–39.