Original article

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Myocardial fibrosis by late gadolinium enhancement cardiac magnetic resonance and hepatitis C virus infection in thalassemia major patients

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Aims **Our aim was to evaluate the correlation between myocardial fibrosis detected using the late gadolinium enhancement (LGE) cardiovascular magnetic resonance (CMR) technique and chronic hepatitis C (CHC) in a large, retrospective, multicentre cohort of thalassemia major patients.**

Methods **LGE images were acquired in 434 thalassemia major patients (233 men, 31 ± 9 years) enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) study. Hepatitis C virus (HCV)-RNA tests were sensitive to detect more than 50 copies/ml.**

Results **No patient manifested moderate/severe adverse events associated with the use of Gadobutrol. Myocardial fibrosis was detected in 90 (21%) patients. Among the 312 patients tested for HCV-RNA, there was a significant correlation between the presence of myocardial fibrosis and CHC ($P = 0.011$). Among the 62 patients with myocardial fibrosis tested for HCV-RNA, we found a significantly higher prevalence of diabetes mellitus in CHC patients versus the no-CHC patients ($P = 0.049$).**

Conclusion **Our findings support the use of the LGE CMR approach well tolerated in the thalassemia major patients with CHC. HCV infection can be involved in the pathogenesis of myocardial fibrosis through both myocarditis directly and the pancreas and liver damage with the development of diabetes indirectly. These patients could therefore benefit from cardioactive drugs and therapeutic interventions directed towards the eradication of virus.**

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Introduction

Thalassemia is the most common genetic disorder worldwide. Although the survival of thalassemia major patients has improved in recent years, heart failure remains the main cause of morbidity and mortality in this population.[1] The pathogenesis of the thalassemic cardiomyopathy has not yet been fully elucidated. Although cardiac siderosis is assumed to be the major cause of heart failure, the high cardiac output state,[2] myocarditis,[3] pulmonary hypertension, endocrinopaties[4] and HCV-induced cardiomyopathy[5] may also play a role. In fact, in thalassemia major patients, left ventricular ejection fraction (LVEF) declined progressively as iron burden increased, but we found patients with abnormal heart function and no significant myocardial iron.[6]

The value of cardiovascular magnetic resonance (CMR) in the management of thalassemia patients is universally accepted.[7] T2* CMR, with a single measurement in the mid-ventricular septum or with a multislice approach, has been validated for quantitative evaluation of myocardial iron overload (MIO).[8] CMR also provides the opportunity to quantify biventricular function parameters with excellent reproducibility. Moreover, cardiac fibrosis can be studied using the late gadolinium enhancement (LGE) CMR technique, which has been demonstrated to be well tolerated also in patients with hemoglobinopaties.[9] LGE has been widely used to examine the role of replacement macroscopic fibrosis in a wide range of cardiomyopathies[10] and the occurrence of LGE is associated with the development of cardiac events.[11] In thalassemia

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major patients, myocardial fibrosis demonstrated histologically[12] has been confirmed using the LGE technique.[4,5,13,14]

In the Italian thalassemia major population, myocardial fibrosis was shown to be a relatively common finding (20%) and it was a time-dependent process not found before 10 years.[4,15] The presence of macroscopic myocardial fibrosis was positively correlated with cardiovascular risk factors, cardiac complications and serum antihepatitis C virus (HCV) antibodies.[5] In thalassemia major patients with diabetes mellitus, the risk for myocardial fibrosis seems to be significantly higher in patients who were positive for both diabetes mellitus and HCV-RNA.[4]

Transfusion-acquired HCV infection is common in thalassemia.[4,5] Clinical studies have indicated a relationship between HCV infection and the development of myocarditis and hypertrophic or dilated cardiomyopathy. Although the mechanisms by which this virus damages the myocardium have not been elucidated,[15–19] a possible role of HCV-infected mononuclear cells was suggested.[20,21]

Our study aimed to retrospectively verify the correlation between myocardial fibrosis detected by LGE CMR and chronic hepatitis C (CHC) in a large cohort of thalassemia major patients.

Materials and methods

Study population

We analysed 434 thalassemia major patients (233 men, mean age 31 ± 9 years) consecutively enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) study. The MIOT project is a network of nine CMR centres and 68 thalassemia centres wherein CMR examinations are performed using homogeneous, standardized and validated procedures and wherein patients' clinical-instrumental data are collected in a centralized database via the world wide web.[22,23] All clinical and laboratory investigations were carried out at the thalassemia centres wherein the patients were treated.

All patients had been regularly transfused since early childhood and began chelation therapy from the mid-to-late 1970s, although patients born after the seventies received chelation therapy from early childhood.

The historical diagnosis of CHC was based on a positive HCV-RNA or on the negative HCV-RNA in patients who had been treated with alpha-interferon (IFN) obtaining a sustained virological response (SVR). In fact, in all SVR patients, the HCV-RNA was positive for at least 6 months with active viral replication and consequent CHC.

The study complied with the Declaration of Helsinki. All patients gave written informed consent to the study. The project was approved by the institutional ethics committee.

Serological and virological examination

The detection of anti-HCV antibodies and of HCV-RNA was performed by different commercial available tests. HCV-RNA qualitative or quantitative tests were sensitive enough to detect more than 50 copies/ml (or UI/ml).[24]

Cardiovascular magnetic resonance

CMR was performed using a 1.5-T magnetic resonance (MR) scanner (GE Signa/Excite, Milwaukee, Wisconsin, USA). In brief, for the MIO measurements, a multislice multiecho T2* approach was used.[25,26] 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 left ventricular model according to the AHA/ACC.[27] The global heart T2* value was obtained by averaging all segmental T2* values. The intraobserver, interobserver and interstudy variability[25] as well as the transferability[23] of the methodology had been previously assessed.

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 to assess biventricular function parameters quantitatively in a standard way, using MASS software (Medis, Leiden, The Netherlands). The intercentre variability for the quantification of cardiac function had been previously reported.[6]

LGE images were acquired in short-axis views from 10 to 18 min, after intravenous administration of Gadobutrol (Gadovist; Bayer Schering Pharma; Berlin, Germany) (0.2 mmol/kg), using a fast gradient-echo inversion recovery sequence. Depending on the LV size, 10–14 short-axis views were acquired. Also, vertical, horizontal and oblique long axis views were acquired. Inversion times were adjusted to null for the normal myocardium (from 210 to 300 ms) with voxel size of $1.6 \times 1.25 \times 8.0$ mm. The LGE extent was evaluated visually by experienced observers using a two-point scale (LGE absent or present). LGE was considered present if visualized in two different views.[5] The extent of LGE areas was quantified using a semiautomatic, previously validated software.[5,28] In each segment through the left ventricular wall, ischemic LGE was defined transmural when the extent of LGE was more than 50% or subendocardial when the extent of LGE was 50% or less. The nonischemic LGE was conventionally classified as mid-wall (patchy, linear or junctional) or epicardial.[29] LGE images were not acquired in patients with a glomerular filtration rate (GFR) less than 30 ml/min/1.73 m^2 and in patients who refused the contrast medium administration.

Statistical analysis

All data were analysed using SPSS version 13.0 statistical packages. All continuous variables were expressed as

mean ± standard deviation. Categorical variables were expressed as frequencies and percentages. Comparisons between groups were made by independent-samples *t*-test or Mann–Whitney test. Chi-square test was performed for noncontinuous variables. A two-tailed probability value of 0.05 was considered statistically significant.

Results

Figure 1 represents the flow chart of the test results in the 434 thalassemia major patients.

Our large cohort of multitransfused thalassemia major patients showed a high prevalence (75%) of HCV infection.

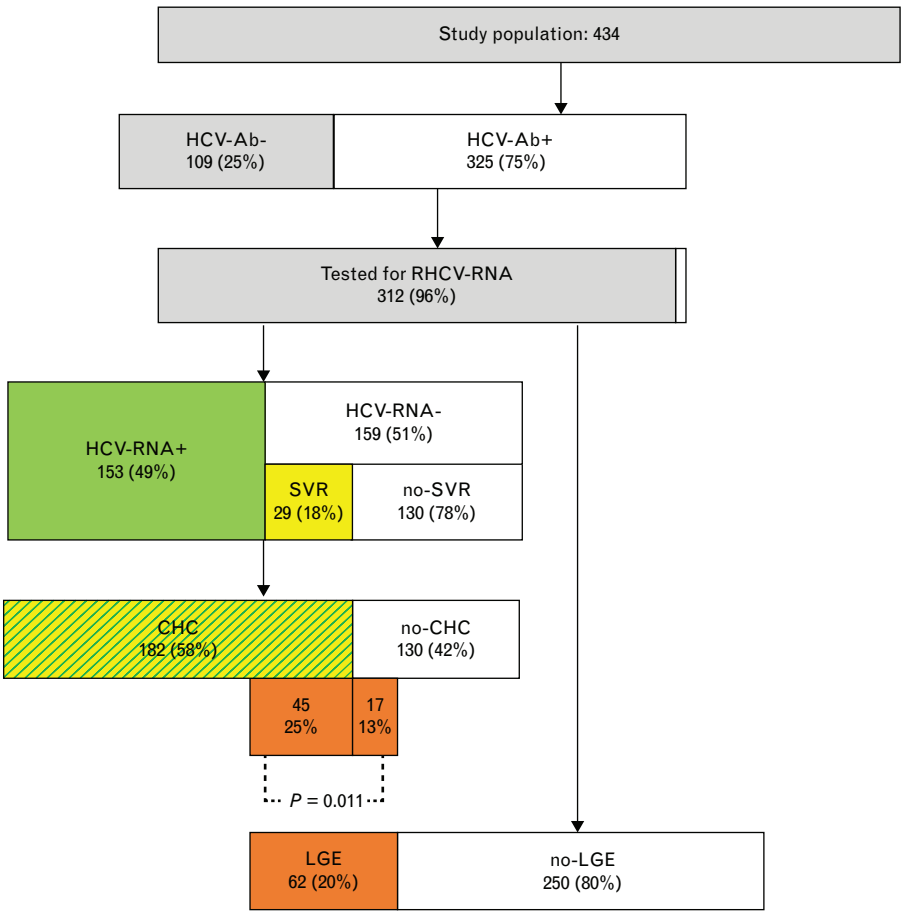
No patient manifested moderate/severe adverse events associated with the use of Gadobutrol. We identified LGE areas in 90 patients. LGE pattern was nonischemic in 89 out of 90 (99%) patients, always with a mid-wall distribution (linear in 93% of cases and junctional in 7% of cases) (Fig. 2). LGE followed a subendocardial ischemic pattern typical of the coronary artery disease in only one patient (1%) HCV-RNA negative and no diabetic. The extent of LGE areas was 1.79 ± 1.39% of the total left myocardial mass. The mean number of LGE segments per patient was 2.02 ± 1.15. Of the 174 areas of fibrosis, 140 (80.5%) involved the septal region, in particular at the mid-level (77 areas).

We reported the more relevant clinical and instrumental findings in the fibrosis and nonfibrosis groups among the 434 patients tested for HCV infection in Table 1.

In Table 2, we showed the more relevant clinical and instrumental findings between CHC and no-CHC groups among the 312 patients tested for HCV-RNA.

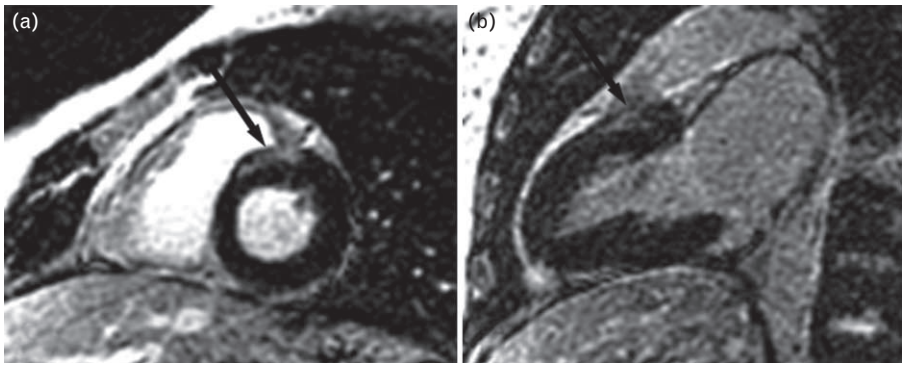
We found a significantly higher prevalence of myocardial fibrosis in the CHC group versus the no-CHC group ($P = 0.011$). The extent of LGE areas with respect to

Fig. 1



Flow chart of the test results. Among the 434 TM patients examined for presence of anti-HCV antibodies, 325 (75%) were positive. Of them, 312 were tested for HCV-RNA and 153 (49%) were positive. Among the 159 patients negative for HCV-RNA, 29 (18%) have become negative after antiviral treatment (patients with SVR or Sustained Virological Response). Thus, 182 patients experienced a CHC (153 positive HVC RNA patients and 29 SVR patients). Presence of LGE was assessed in the 312 patients tested for HCV-RNA, with a 20% (62 patients) incidence of LGE. Presence of LGE was significantly higher in CHC group with respect to no-CHC group ($P = 0.011$).

Fig. 2



Thalassemia major hepatitis C virus-RNA positive patient with epi-mesocardial delayed enhancement area (black arrows) in the antero-septal junction visualized in the short-axis view (a) and in the oblique plane (b), acquired from the short-axis view on the enhancement area.

the total left myocardial mass was comparable between the two groups (CHC $1.91 \pm 1.61\%$ versus no-CHC $1.39 \pm 0.68\%$, $P = 0.812$). In both groups, the septal region was more frequently involved, in particular at the mid-level ($P = 0.674$). All CHC patients showed LGE with an epi-mesocardial pattern. The presence of LGE areas in the infero-septal or antero-septal junctions was significantly lower in the CHC group versus the no-CHC group (27.3 versus 58.8%; $P = 0.021$). We did not find significant correlation between the presence of LGE and the active viral replication: in the LGE group, 36 out of 62 patients were positive for HCV-RNA, in no-LGE 117/250 patients were positive for HCV-RNA ($P = 0.112$).

The prevalence of the LGE areas was comparable between the HCV-RNA positive patients and the SVR patients [36/153 (24%) versus nine out of 29 patients (31%); $P = 0.573$].

Among the 62 patients with myocardial fibrosis tested for HCV-RNA, we found a significantly higher prevalence of diabetes mellitus in CHC patients versus the no-CHC patients (10/45 patients versus 0/17 patients; $P = 0.049$). All patients had developed the diabetes mellitus after HCV infection.

Discussion

In this large, multicentre study on a cohort of Italian thalassemia major patients, the high frequency of positive anti-HCV antibodies is concordant with previously reported data.[5] The prevalence of myocardial fibrosis detected by the LGE CMR technique was comparable with previous works on Italian thalassemia major patients[4,5,13] and the LGE CMR approach has been confirmed as well tolerated. In our large Italian cohort, the prevalence of myocardial fibrosis was significantly higher (21%) than that detected in the study of Kirk *et al.*,[14] wherein out of 45 thalassemia major patients only one with positive HCV antibodies showed LGE. The significantly higher prevalence of positive HCV antibodies in this Italian thalassemia major population versus the English thalassemia major study group[14] (75 versus

Table 1 More relevant clinical and instrumental findings in the myocardial fibrosis group and nonfibrosis group among the 434 patients tested for hepatitis C virus infection

	Fibrosis group ($n = 90$)	Nonfibrosis group ($n = 344$)	*P*
Age (years)	31.88 ± 8.52	30.64 ± 8.86	0.233
Sex (M/F)	43/47	171/173	0.744
Mean pretransfusional haemoglobin (g/dl)	9.74 ± 1.29	9.63 ± 0.56	0.252
Serum ferritin (ng/dl)	1397.84 ± 1173.74	1693.52 ± 1623.26	0.267
ALT (units/l)	45.17 ± 31.51	47.45 ± 38.15	0.665
Diabetes mellitus (%)	11/76 (14.5%)	35/269 (13%)	0.740
Obesity (%)	1.1	2.9	0.473
BMI (kg/m^2)	22.59 ± 2.91	23.98 ± 28.74	0.335
HCV-Ab			
Positive	66	259	0.70
Negative	24	85	
LVEF (%)	60.64 ± 8.05	61.52 ± 7.26	0.318
RVEF (%)	59.79 ± 8.73	59.50 ± 7.70	0.762
LV EDVI (ml/m^2)	94.98 ± 20.80	88.00 ± 18.82	0.002
RV EDVI (ml/m^2)	91.74 ± 22.91	86.65 ± 23.10	0.061
Global heart T2* (ms)	26.72 ± 14.05	26.84 ± 12.87	0.815

ALT, amino alanine transferase; LVEF, left ventricular ejection fraction.

Table 2 More relevant clinical and instrumental findings in the chronic hepatitis C group and nonchronic hepatitis C group among the 312 patients tested for hepatitis C virus RNA

	CHC group ($n=182$)	No-CHC group ($n=130$)	P
Age (years)	34.19 ± 6.50	33.11 ± 6.95	0.199
Sex (M/F)	89/93	61/69	0.730
Mean pretransfusional haemoglobin (g/dl)	9.55 ± 0.53	9.70 ± 0.63	0.023
Serum ferritin (ng/dl)	1381.65 ± 1220.72	1437.95 ± 1612.99	0.574
ALT (units/l)	61.22 ± 44.76	34.98 ± 25.41	<0.0001
Diabetes mellitus (%)	17.3	13.7	0.442
Obesity (%)	1.6	4.6	0.171
BMI (kg/m^2)	22.74 ± 3.01	23.13 ± 3.35	0.521
Myocardial fibrosis (%)	24.7	13.1	0.011
LVEF (%)	60.93 ± 8.15	62.03 ± 6.88	0.182
RVEF (%)	59.55 ± 8.65	59.24 ± 8.14	0.705
LV EDVI (ml/m^2)	90.61 ± 20.68	86.81 ± 17.59	0.072
Global heart T2* (ms)	27.40 ± 12.54	27.19 ± 14.15	0.778

ALT, amino alanine transferase; CHC, chronic hepatitis C; LVEF, left ventricular ejection fraction.

22%) might resolve the differences in the LGE prevalence.

As previously retrospectively reported,[5,13,30] we confirmed the lack of correlation between myocardial fibrosis and heart iron in this multicentre cohort of well transfused and well chelated thalassemia major patients. Conversely, myocardial fibrosis seems to have a negative impact on the ventricular remodelling considering the significantly higher end-diastolic volume indexes in the fibrosis group.

A previous study on a smaller cohort of thalassemia major patients has reported a significant association between the presence of myocardial LGE areas and anti-HCV antibodies.[5] We were unable to confirm this datum in our larger cohort of thalassemia major patients. However, the correlation observed in our series between the diagnosis of CHC and the myocardial fibrosis supports the hypothesis of a role for HCV infection as a possible cause of myocarditis and consequent myocardial fibrosis. In fact, patients with only positive HCV antibodies do not constantly experience persistent virus C replication that can be responsible for myocarditis.

Our patients with CHC showed significantly higher amino alanine transferase (ALT) levels correlated with the liver disease. The induced-emolysis by the antiviral therapy ribavirin could justify the mean pretransfusional haemoglobin levels significantly lower in the CHC group. The relationship between the CHC patients and the presence of LGE areas with an epi-mesocardial pattern suggests that HCV infection could be involved in the pathogenesis of myocardial fibrosis by the development of myocarditis,[16,17] in particular in thalassemia major wherein the immune system deficit is well known.[1] Indeed, areas of LGE representing myocardial damage have been frequently detected in patients with acute/chronic myocarditis.[11] All our CHC patients showed LGE with an epi-mesocardial pattern. In the CHC group, we found a significantly lower presence of LGE areas in the junctions more associated with pressure-overload states than myocarditis itself.

The extent of LGE areas was comparable between CHC and no-CHC group and it was not high. However, our preliminary prospective data seem to identify this confined myocardial fibrosis as an independent prognosticator for heart failure in thalassemia major.[31]

Although the high prevalence (31%) of LGE areas in the SVR group of patients treated with alpha-interferon could indicate that the drug induces myocardial fibrosis, to date, such an effect of IFN has not been reported. Moreover, the prevalence of the LGE areas was comparable between the HCV-RNA positive patients and the SVR patients ($P=0.573$). On the contrary, in a Japanese study, treatment with IFN resulted in disappearance of myocardial abnormalities as demonstrated by thallium scintigraphy.[32] On the contrary, it can be speculated that HCV infection contributes to the development of a clinically silent myocarditis causing myocardial fibrosis.

Moreover, among the patients with myocardial fibrosis, we found a significantly higher prevalence of diabetes mellitus in patients with CHC. Similarly, in a previous study on a large retrospective historical cohort of thalassemia major wherein diabetes mellitus was associated with a higher risk of heart complications, the risk for myocardial fibrosis by LGE was significantly higher in patients who were positive for both diabetes mellitus and HCV-RNA.[4] These findings support the hypothesis that HCV infection produces LGE through not only myocarditis directly but also the pancreas and liver damage with the development of diabetes indirectly. Diabetes mellitus can reinforce the oxidative stress and cardiac microvascular angiopathy produced by HCV infection. In fact, in the diabetes mellitus population, myocardial fibrosis has been documented by the LGE technique due to micro and macro-angiopathy.[33]

In the literature, only a few, controversial reports flawed by methodological problems have addressed the relationship between HCV infection and cardiac damage. To evaluate the possible association between HCV and

idiopathic dilated cardiomyopathy, Dos Reis *et al.*[34] performed a systematic review of the literature for the years 1995–2005. Only six were methodologically in accordance with the standards of the review and a significant association between cardiomyopathy and HCV was found in only two Japan studies by the same author. Out of 106 autoptic hearts from patients with myocarditis and cardiomyopathy, HCV-RNA was detected in 13 hearts; in four of them (33%), there were signs of myocarditis.[18] In a more recent article by the same author, exploring the prevalence of HCV infection among patients with myocarditis and heart failure, anti-HCV antibodies were identified in 59 of 1355 stored sera and they were more prevalent than in the general population (4.4 versus 1.8%).[17] In another series, out of 48 patients with myocarditis, three (6%) had anti-HCV antibodies. A lymphocytic myocarditis and presence of HCV-RNA in serum and myocardium were shown,[35] suggesting that HCV also replicated in myocardium contributing to the development of myocarditis.

Thus, HCV infection may be an important cause of myocarditis and consequently of macroscopic myocardial fibrosis in politransfused patients, in whom the prevalence of HCV infection is high.

Despite the inclusion of HCV among the cardiotropic viruses, the mechanisms by which this virus damages the myocardium have not been elucidated. One hypothesis is that the HLA and non-HLA systems are implicated in the susceptibility to the development of HCV-associated cardiomyopathy.[35] Omura *et al.*[36] supported the hypothesis of anatomic damage in myocardium by direct activity of the hepatitis C virus by an experimental study in transgenic mice for the HCV-core gene.

The concept that, in selected patients, cardiomyopathy could be seen as an extrahepatic manifestation of HCV infection is of great importance for the treatment. Antiviral therapy available for chronic hepatitis C is at present considered contraindicated in patients with myocardial dysfunction and, when including ribavirin, partially contraindicated in transfusion-dependent patients increasing the need for blood consumption.

Some limitations could be recognized in the study. The data were cross-sectionally analysed from a prospectively accumulated database. LGE was adopted as an indicator of fibrosis as endomyocardial biopsy, which would be the 'gold standard' for fibrosis assessment, was not indicated in the majority of the study population. It is possible that the resolution of LGE imaging is insufficient to visualize diffuse myocardial fibrosis resulting from HCV 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 setting of patients would be the use of CMR T1 mapping techniques for the left ventricular myocardium.

Conclusion

In a large multicentre cohort of thalassemia major patients cross-sectionally, we found a significant correlation between the presence of myocardial fibrosis and chronic hepatitis C. These findings confirm the importance of completing the cardiac CMR with a well tolerated LGE approach in order to evaluate macroscopic myocardial fibrosis in thalassemia major patients with CHC. In fact, our data seem to support a possible role of the HCV chronic infection in the pathogenesis of the myocardial fibrosis in multitransfused thalassemia major patients through both myocarditis directly and the pancreas and liver damage with the development of diabetes indirectly. These patients could therefore benefit from cardioactive drugs and therapeutic interventions directed towards the eradication of the virus.

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Conflicts of interest

There are no conflicts of interest.

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