Increased myocardial extracellular volume is associated with myocardial iron overload and heart failure in thalassemia major

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Abstract

Objectives Myocardial extracellular volume (ECV) by cardiovascular magnetic resonance (CMR) is a surrogate marker of diffuse fibrosis. We evaluated the association between ECV and demographics, CMR findings, and cardiac involvement in patients with thalassemia major (TM).

Methods A total of 108 β-TM patients (62 females, 40.16 ± 8.83 years), consecutively enrolled in the Extension-Myocardial Iron Overload in Thalassemia Network, and 16 healthy subjects (6 females, 37.12 ± 16.13 years) underwent CMR. The protocol included assessment of T2*, native T1, and T2 values in all 16 myocardial segments for myocardial iron overload (MIO) quantification, cine images for left ventricular (LV) function quantification, post-contrast T1 mapping for ECV calculation, and late gadolinium enhancement (LGE) technique for replacement myocardial fibrosis detection.

Results Global ECV values were significantly higher in females than in males. Global ECV values were significantly higher in patients with significant MIO (global heart T2* < 20 ms) than in patients without significant MIO, and both groups exhibited higher global ECV values than healthy subjects. No association was detected between native T1 and ECV values, while patients with reduced global heart T2 values showed significantly higher global ECV values than patients with normal and increased global heart T2. Global ECV values were not correlated with LV function/size and were comparable between patients with and without LGE. Compared to patients without heart failure, patients with a history of heart failure ($N = 10$) showed significantly higher global heart ECV values.

Conclusion In TM, increased myocardial ECV, potentially reflecting diffuse interstitial fibrosis, is associated with MIO and heart failure.

Key Points

- *CMR-derived myocardial extracellular volume is increased in thalassemia major patients, irrespective of the presence of late gadolinium enhancement.*
- *In thalassemia major, myocardial iron overload contributes to the increase in myocardial ECV, which potentially reflects diffuse interstitial fibrosis and is significantly associated with a history of heart failure.*

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Keywords Thalassemia · Magnetic resonance imaging · Iron overload · Fibrosis · Heart failure

Abbreviations

CMR	Cardiac magnetic resonance
CoV	Coefficient of variation
DIF	Diffuse interstitial fibrosis
ECV	Extracellular volume fraction
E-MIOT	Extension-Myocardial Iron Overload in Thalassaemia
GRE	Gradient-echo
HCV	Hepatitis C virus
HF	Heart failure
LGE	Late gadolinium enhancement
LIC	Liver iron concentration
LV	Left ventricle
MEFSE	Multi-echo fast-spin-echo
MIO	Myocardial iron overload
MOLLI	Modified look-locker inversion recovery
NT-proBNP	N-terminal fragment of B-type natriuretic peptide
SD	Standard deviations
TM	Thalassemia major

Introduction

Cardiovascular magnetic resonance (CMR) is the gold standard for the non-invasive assessment of myocardial tissue properties [1]. T2* CMR has been used for two decades as a non-invasive tool for quantifying myocardial iron overload (MIO), offering the possibility to tailor and monitor iron chelation therapies [2]. MIO is a particular concern in patients with thalassemia major (TM), who require lifelong regular blood transfusions for survival [3]. T2* CMR has gained a pivotal role in the risk stratification of TM patients [4, 5] and has opened their prognosis [6, 7].

Late gadolinium enhancement (LGE) CMR allowed the non-invasive detection of focal/replacement myocardial fibrosis. Replacement myocardial fibrosis was demonstrated to be a relatively common finding among adult TM patients, predicting heart failure (HF) and cardiac complications [5]. Corresponding to the collagen deposition that occurs following myocyte apoptosis/necrosis [8], replacement fibrosis represents a relatively late stage of the disease, likely not reversible. Diffuse interstitial fibrosis (DIF), representing the diffuse, disproportionate accumulation of collagen in the interstitial space within myocytes, occurs earlier in the course of the disease. It may be missed in conventional LGE imaging, which relies on the difference in signal intensity between normal nulled and fibrotic myocardium and has a limited spatial resolution [9]. This limitation has been overcome by the CMR T1-mapping techniques, allowing to assess the extracellular volume fraction (ECV). In the absence of amyloid deposition or edema, increased ECV results from increased myocardial collagen fraction and is a good surrogate imaging marker of DIF [1]. CMR-derived ECV was demonstrated to be well correlated with histologically quantified myocardial collagen [10].

According to human histopathological studies [11] and animal models [12], DIF can be present in the setting of MIO. It has been postulated that, despite the intensive chelation treatment, the presence of diffuse myocardial fibrosis may contribute to hamper the HF reversal [9]. Since DIF is believed to be reversible if early, focused treatment is administered [8], its detection may provide new insights into the treatment of iron-induced HF.

To the best of our knowledge, only one study has quantified myocardial ECV in TM patients [13]. A significant increase in myocardial ECV was demonstrated in patients with prior MIO versus patients without prior MIO and healthy controls. No association was detected between ECV and same-day mid-septum T2* values, likely due to the limited sample size and number of patients with evidence of current MIO. Moreover, while the ECV was assessed in all 16 myocardial segments of the left ventricle (LV), the T2* was quantified only in the mid-ventricular septum. The segmental T2* approach is more sensitive, allowing to detect iron overload in different cardiac segments, even in the presence of a normal mid-septum T2* [14]. No data are available about the association between segmental ECV and T2* measurements. Importantly, the link between ECV measurements and cardiac diseases in TM has never been explored.

Hence, this study was conducted to evaluate the association between ECV and T2* values by a segmental approach and the correlation between global ECV values and demographics, CMR findings, and cardiac involvement.

Methods

Study population

We considered 108 β-TM patients (62 females, 40.16 ± 8.83 years), consecutively enrolled in the Extension-Myocardial Iron Overload in Thalassaemia (E-MIOT) project, an Italian network constituted by 66 thalassemia centers and 11 validated MR sites [15].

We enrolled as controls 16 subjects (6 females, 37.12 ± 16.13 years) without hematological or hereditary cardiovascular diseases, symptoms of inflammation, and coronary artery disease. These subjects were referred as patients for a CMR scan, which turned out to be normal.

The study complied with the Declaration of Helsinki and was approved by the local ethical committee. All patients gave written informed consent.

MR

MR exams were performed in the reference MR center of the E-MIOT Network (Pisa) using a 1.5 T scanner (Signa Artist; GE Healthcare). A 30-element cardiac phased-array receiver surface coil with breath-holding and ECG-gating was used.

Three parallel short-axis slices (basal, medium, and apical) of the LV were acquired in end-diastole by a modified look-locker inversion recovery (MOLLI) sequence for T1 mapping [16], a multi-echo gradient-echo (GRE) sequence for T2* assessment [17], and a multi-echo fast-spin-echo (MEFSE) sequence for T2 mapping [18]. MOLLI images were acquired before and 10 min after the intravenous administration of Gadobutrol (Gadovist®; Bayer Schering Pharma) at a dose of 0.2 mmoL/kg. Pixel-wise native and post-contrast T1 maps and T2 maps were generated on the scanner and transferred to a dedicated workstation for offline post-processing that involved manual tracing of endocardial and epicardial borders, with care taken to avoid blood pool and epicardial fat [19]. Basal and medium slices were divided into 6 equiangular segments and the apical slice into 4 segments, according to the AHA/ACC model [20]. The T2 and T1 value in each segment was obtained by averaging the T2 and T1 value, respectively, for all the pixels within the segment. Segmental ECV values were calculated with input of native and post-contrast myocardial segmental and blood pool T1 values and hematocrit assessed on the same day [21]. T2* image analysis was performed using custom-written, validated software (HIPPOMIOT®) [22]. After the calculation of T2* values in all myocardial segments, an appropriate correction map compensated for susceptibility artifacts [23]. Global T1, ECV, T2, and T2* values were obtained by averaging all segmental values.

For hepatic IO assessment, a mid-hepatic slice was obtained by a T2* GRE multiecho sequence [24]. Hepatic T2* values, calculated in a circular region of interest [24], were converted into liver iron concentration (LIC) [25].

Steady-state free precession cine images were acquired in sequential 8-mm short-axis slices from the atrio-ventricular ring to the apex to quantify LV function parameters in a standard way using MASS® software (Medis Medical Imaging) [26].

To detect the presence of replacement myocardial fibrosis, LGE short-axis, vertical, horizontal, and oblique long-axis images were acquired 10–18 min after contrast medium administration. The LGE presence was evaluated visually using a two-point scale (LGE absent or present). LGE was considered present when visualized in two different views [27].

Diagnostic criteria

The value of 20 ms was used as "conservative" normal value for segmental and global heart T2* values [28]. The lower and upper limits of normal for T1/T2 values were calculated on original or log-transformed values measured in 80 healthy subjects as mean ± 2 standard deviations (SD). For global heart T1 values, normal range was 928–1060 ms in males and 989–1085 ms in females. For global heart T2 values, normal range was 48–56 ms in males and 50–57 ms in females.

HF was diagnosed based on symptoms, signs, and instrumental findings according to the current guidelines [29]. Arrhythmias were diagnosed if documented by ECG or 24 hours Holter ECG and if requiring specific medications [30, 31]. In presence of clinical manifestations, the diagnosis of myo/pericarditis required confirmation by cardiac biomarkers, non-invasive imaging modalities, and biopsy where indicated [32]. The term "cardiac complications" included HF, arrhythmias, and myo/pericarditis.

Reproducibility analysis

To evaluate the intra-observer reproducibility, native and post-contrast T1 maps from 20 patients were re-analyzed by the same operator. To evaluate the inter-observer reproducibility, the same images were blindly analyzed by a second operator.

A paired-sample *t*-test or Wilcoxon signed-rank test was applied to detect significant differences between the two datasets. The coefficient of variation (CoV) was obtained as ratio of the SD of the half mean square of the differences between the repeated values, to the general mean. The Bland-Altman technique was used to calculate bias and agreement between two datasets.

Statistical analysis

All data were analyzed using SPSS v.27.0 and MedCalc v.19.8 statistical packages.

Continuous variables were described as mean ± SD and categorical variables were expressed as frequencies and percentages.

The normality of distribution of the parameters was assessed by using the Kolmogorov-Smirnov test or the Shapiro-Wilk test (sample size ≤ 50).

For continuous values with normal distribution, comparisons between groups were made by independent-samples *t*-test (for 2 groups) or one-way ANOVA (for more than 2 groups). Wilcoxon's signed-rank test or Kruskal-Wallis test was applied for continuous values with non-normal distribution. χ^2 testing was performed for non-continuous variables. Bonferroni post hoc test was used for multiple comparisons between pairs of groups.

Correlation analysis was performed using Pearson's test or Spearman's test where appropriate.

A 2-tailed $p < 0.05$ was considered statistically significant.

Results

Intra- and inter-observer variability

Reproducibility results for segmental and global ECV values are summarized in Table 1.

The segmental CoV for the intra-operator reproducibility ranged from 2.89 to 5.36% and the Bland-Altman analysis demonstrated no significant bias.

There was no significant difference between the segmental ECV values obtained by the two operators, with a CoV ranging from 4.50 to 6.83%.

Characteristics of the study population

No difference in terms of sex and age was detected between controls and TM patients ($p = 0.135$ and $p = 0.224$, respectively). Compared to controls, TM patients showed significantly lower global heart T1 values (955.25 ± 89.15 ms vs. 996.25 ± 26.15 ms; $p = 0.034$) (Fig. 1a) and significantly higher global ECV values (32.54 ± 6.59% vs. 25.02 ± 2.65%; $p < 0.0001$) (Fig. 1b).

By definition, all healthy subjects had negative LGE while replacement myocardial fibrosis was detected in 44 (40.7%) TM patients ($p = 0.001$). One patient showed a transmural LGE in the apical inferior segment while the remaining 43 patients had a non-ischemic pattern. The 79.5% of patients had > two foci of fibrosis. When excluding the TM patients with positive LGE, the LGE-negative TM patients showed comparable global heart T1 values versus healthy subjects (976.35 ± 74.71 ms vs. 996.25 ± 26.15 ms; $p = 0.121$) but significantly higher global ECV values (32.26 ± 5.93% vs. 25.02 ± 2.65%; $p < 0.0001$).

Figure 2 displays a LGE-positive patient with normal native and ECV values.

Demographic, clinical, and CMR characteristics of TM patients are summarized in Table 2.

Clinical correlates of ECV in TM

Global ECV values were significantly higher in females than in males (33.88 ± 7.07% vs. 30.74 ± 5.45%; $p = 0.014$) (Fig. 3) but were not correlated with age ($R = -0.037$; $p = 0.704$).

Table 1 Intra- and inter-observer reproducibility data for ECV measurements

Region	Intra-operator				Inter-operator			
	Paired test		Bland-Altman limits (%)	CoV (%)	Paired test		Bland-Altman limits (%)	CoV (%)
	Mean difference (%)	*p*-value			Mean difference (%)	*p*-value		
1: Basal-anterior	−0.37 ± 1.15	0.180	−2.63 to 1.88	2.89	−0.44 ± 2.32	0.423	−4.98 to 4.11	5.61
2: Basal-anteroseptal	−0.19 ± 1.71	0.622	−3.56 to 3.16	3.79	−0.65 ± 2.61	0.290	−5.76 to 4.46	5.88
3: Basal-inferoseptal	−0.11 ± 1.93	0.807	−3.89 to 3.67	4.25	−0.22 ± 2.33	0.690	−4.79 to 4.36	5.03
4: Basal-inferior	−0.29 ± 1.38	0.371	−2.99 to 2.42	3.19	0.75 ± 4.17	0.443	−7.42 to 8.92	4.59
5: Basal-inferolateral	−0.31 ± 2.29	0.562	−4.80 to 4.18	5.01	0.27 ± 2.71	0.677	−5.04 to 5.56	4.50
6: Basal-anterolateral	−0.23 ± 2.03	0.213	−4.21 to 3.74	4.69	−0.89 ± 3.09	0.214	−6.94 to 5.16	6.53
7: Medium-anterior	0.38 ± 2.51	0.518	−4.54 to 5.30	5.36	−0.19 ± 3.09	0.803	−6.25 to 5.88	6.61
8: Medium-anteroseptal	−0.58 ± 2.34	0.283	−5.17 to 4.00	4.92	−0.51 ± 2.55	0.396	−5.50 to 4.48	5.34
9: Medium-inferoseptal	−0.04 ± 2.05	0.929	−4.07 to 3.98	4.49	−0.59 ± 2.41	0.308	−5.31 to 4.12	5.42
10: Medium-inferior	0.03 ± 1.89	0.939	−3.67 to 3.74	4.17	−0.18 ± 2.29	0.745	−4.66 to 4.31	4.86
11: Medium-inferolateral	−0.31 ± 2.26	0.552	−4.74 to 4.12	5.00	−0.42 ± 2.71	0.500	−5.72 to 4.89	5.99
12: Medium-anterolateral	0.16 ± 2.15	0.737	−4.05 to 4.38	4.39	0.19 ± 2.15	0.696	−4.02 to 4.41	4.39
13: Apical-anterior	−0.63 ± 2.29	0.272	−5.12 to 3.85	4.85	−0.98 ± 3.41	0.241	−7.67 to 5.71	6.54
14: Apical-septal	−0.28 ± 2.15	0.581	−4.49 to 3.93	4.29	0.20 ± 3.02	0.784	−5.71 to 6.12	6.11
15: Apical-inferior	0.35 ± 2.37	0.540	−4.29 to 4.98	4.99	−0.12 ± 3.19	0.884	−6.37 to 6.13	6.83
16: Apical-lateral	−0.22 ± 2.14	0.668	−4.42 to 3.98	4.37	−0.66 ± 4.62	0.550	−9.72 to 8.39	6.22
Global	−0.19 ± 1.24	0.483	−2.63 to 2.23	2.65	−0.17 ± 2.28	0.821	−4.58 to 4.35	4.82

CoV coefficient of variability

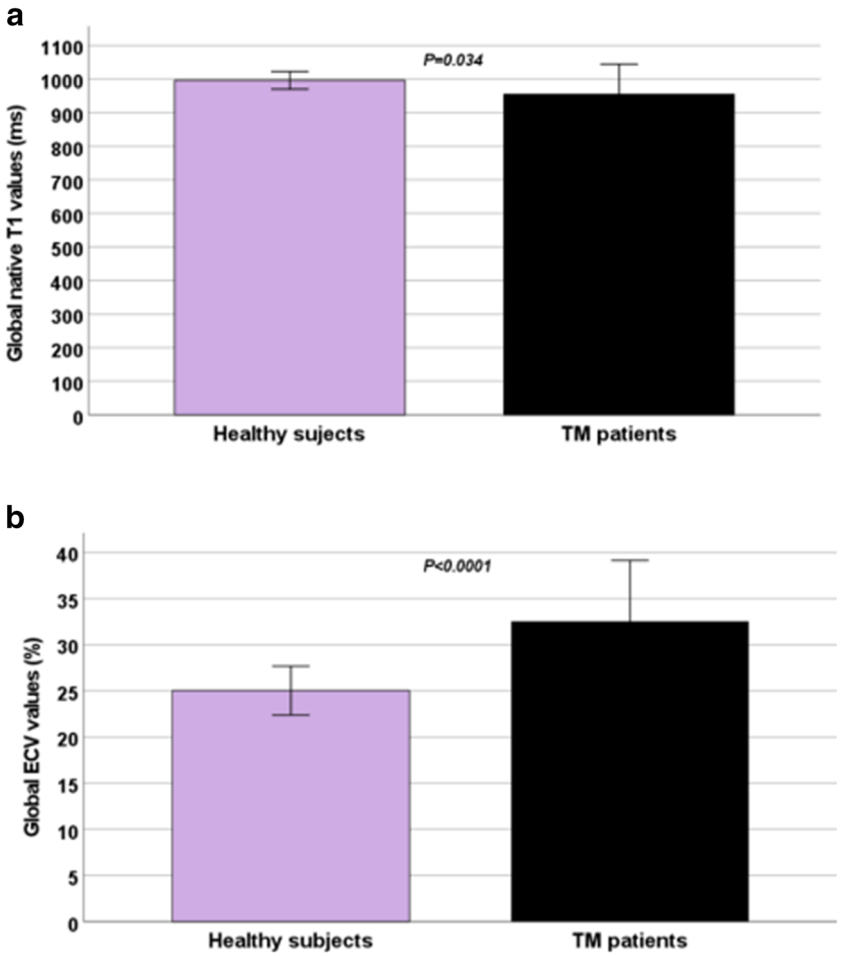

Fig. 1 Comparison of global native (**a**) and ECV (**b**) values between healthy subjects and TM patients

Global ECV values were comparable between patients with and without the spleen (32.77 ± 6.48% vs. 32.29 ± 6.75%; $p = 0.711$).

The group including patients who never contracted the hepatitis C virus (HCV) infection and who spontaneously cleared the virus in the first 6 months of infection and the group constituted by patients who eradicated the virus after the treatment with antiviral therapy and by those with chronic HCV infection showed comparable global ECV values (32.19 ± 5.87% vs. 33.20 ± 7.84%; $p = 0.453$). Note that both patients with chronic HCV infection had an increased global ECV (38% and 41%).

Serum ferritin levels were not significantly correlated with global ECV but showed a significant inverse correlation with global heart T2* ($R = -0.541$; $p < 0.0001$), global heart T1 ($R = -0.407$; $p < 0.0001$), and global heart T2 ($R = -0.274$; $p = 0.014$). Global ECV values were not correlated with serum hemoglobin and high sensitivity cardiac troponin T levels but were positively correlated with N-terminal fragment of B-type natriuretic peptide (NT-proBNP) levels ($R = 0.237$; $p = 0.016$).

Patients without and with at least one cardiovascular risk factor showed comparable global ECV values (31.89 ± 5.65% vs. 33.00 ± 7.46%; $p = 0.404$).

ECV and MR parameters

MRI LIC values were positively correlated with global heart T2* values ($R = -0.487$; $p < 0.0001$), global heart T1 values ($R = -0.232$; $p < 0.0001$), and global heart T2 values ($R = -0.468$; $p < 0.0001$), and negatively correlated with global ECV values ($R = 0.197$; $p = 0.041$).

Significant MIO (global heart T2* < 20 ms) was found in 9 (8.3%) patients. Global ECV values were significantly higher in patients with significant MIO than in patients without significant MIO (40.07 ± 10.62% vs. 31.85 ± 5.71%; $p = 0.049$) (Fig. 4a) while no significant difference was detected in the hematocrit level (31.63 ± 2.84% vs. 32.14 ± 3.41%; $p = 0.664$). Both groups of patients without and with significant MIO exhibited higher global ECV values compared to healthy subjects ($p < 0.0001$ for both comparisons). The ECV value was not available for 63 segments, due to the presence of

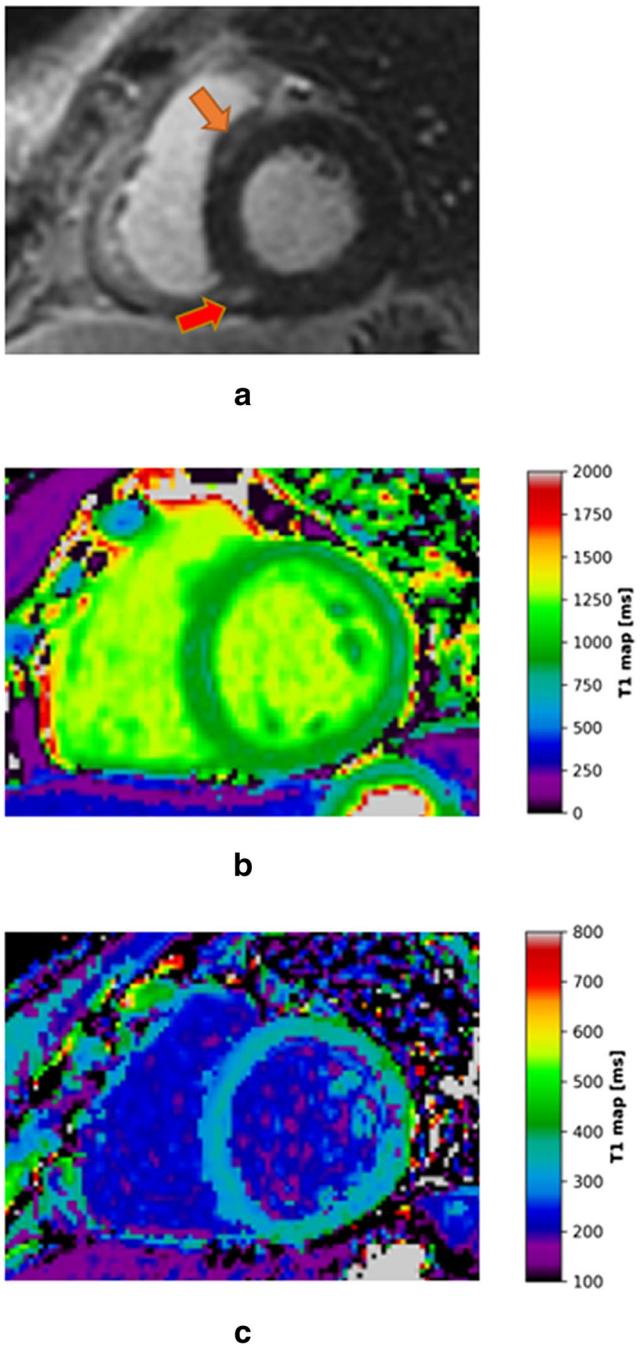

Fig. 2 CMR of a 46-year-old female patient with TM: basal short axis LGE image (**a**) and T1 maps acquired pre-contrast (**b**) and post contrast (**c**). The patient showed intramural enhancement in anterior septum (orange arrow) and junctional spotty LGE at inferior junction (red arrow), normal native T1 in all myocardial segments (T1 = 906 ms in anterior septum), and normal ECV (26%)

Table 2 Demographic, clinical, and MRI data of TM patients

Variable	Value
Demographics and clinical features	
Sex (males/females)	46/62
Age (years)	40.16 ± 8.83
Transfusion starting age (months)	20.42 ± 19.82
Chelation starting age (years)	4.32 ± 4.35
Splenectomy, N (%)	53 (49.1)
HCV infection, N (%)	
Negative	43 (39.8)
Spontaneously cleared	28 (25.9)
Eradicated after therapy	35 (32.4)
Positive	2 (1.9)
Body surface area (m^2)	1.59 ± 0.17
Biochemical profile	
Pre-transfusion hemoglobin (g/dl)	9.75 ± 0.47
Serum ferritin (ng/l)	1182.83 ± 1796.66
Hematocrit (%)	32.09 ± 3.35
NT-proBNP (ng/l)	159.02 ± 199.84
High sensitivity cardiac troponin T (ng/l)	6.16 ± 4.28
Cardiovascular risk factors	
Family history, N (%)	12/96 (12.5)
Current or former smoking, N (%)	20/103 (19.4)
Hypertension, N (%)	9 (8.3)
Dyslipidemia, N (%)	4 (3.7)
Diabetes, N (%)	18 (16.7)
Alcohol abuse, N (%)	1/106 (0.9)
Obesity, N (%)	6 (5.6)
At least one CVRF, N (%)	52/100 (52.0)
Magnetic resonance	
Global heart T2* (ms)	37.51 ± 9.51
Global heart T1 (ms)	955.25 ± 89.15
Global ECV (%)	32.54 ± 6.59
Global heart T2 (ms)	55.85 ± 5.26
LV end-diastolic volume index (ml/m^2)	84.59 ± 16.07
LV end-systolic volume index (ml/m^2)	31.97 ± 10.34
LV stroke volume index (ml/m^2)	52.39 ± 9.14
LV mass index (g/m^2)	60.89 ± 13.22
LV ejection fraction (%)	62.67 ± 7.31
Replacement myocardial fibrosis, N (%)	44 (40.7)
MRI LIC (mg/g dw)	7.56 ± 11.42

N number, *HCV* hepatitis C virus, *NT-proBNP* N-terminal fragment of B-type natriuretic peptide, *CVRF* cardiovascular risk factor, *ECV* extracellular volume, *LV* left ventricular, *MRI* magnetic resonance imaging, *LIC* liver iron concentration

significant artifacts in T1 images. Out of the considered 1665 segments, 145 (8.71%) had a T2* < 20 ms. Segments with pathological T2* had significantly higher ECV values than segments with normal T2* (37.49 ± 12.17% vs. 31.84 ± 7.64%; $p < 0.0001$) (Fig. 4b).

Global heart T1 values were normal in 54 (50%) patients, reduced in 50 (46.3%) patients and increased in 4 (3.7%) patients. Global ECV tended to be higher among patients with increased T1 value (37.13 ± 4.46%) versus both patients with

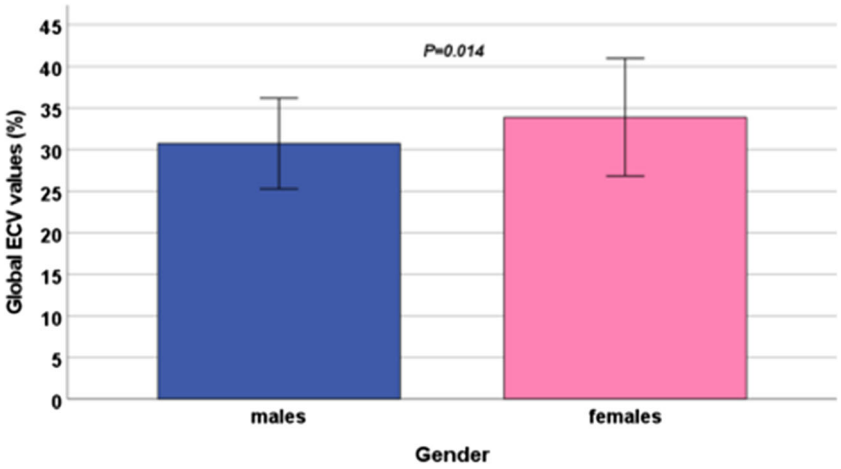

Fig. 3 Association between global ECV values and gender in TM patients

normal or reduced T1 (31.84 ± 6.52% and 32.93 ± 6.7%, respectively), but the difference among the three groups was not significant ($p = 0.259$).

MEFSE images were acquired in 90 patients, of whom 40 (44.4%) had a normal global heart T2 value, 9 (8.3%) a reduced global heart T2 value, and 41 (45.6%) an increased global heart T2 value. Patients with reduced global heart T2 value showed significantly higher global ECV values than patients with normal (40.25 ± 9.74% vs. 30.54 ± 5.49%; $p < 0.0001$) as well as increased

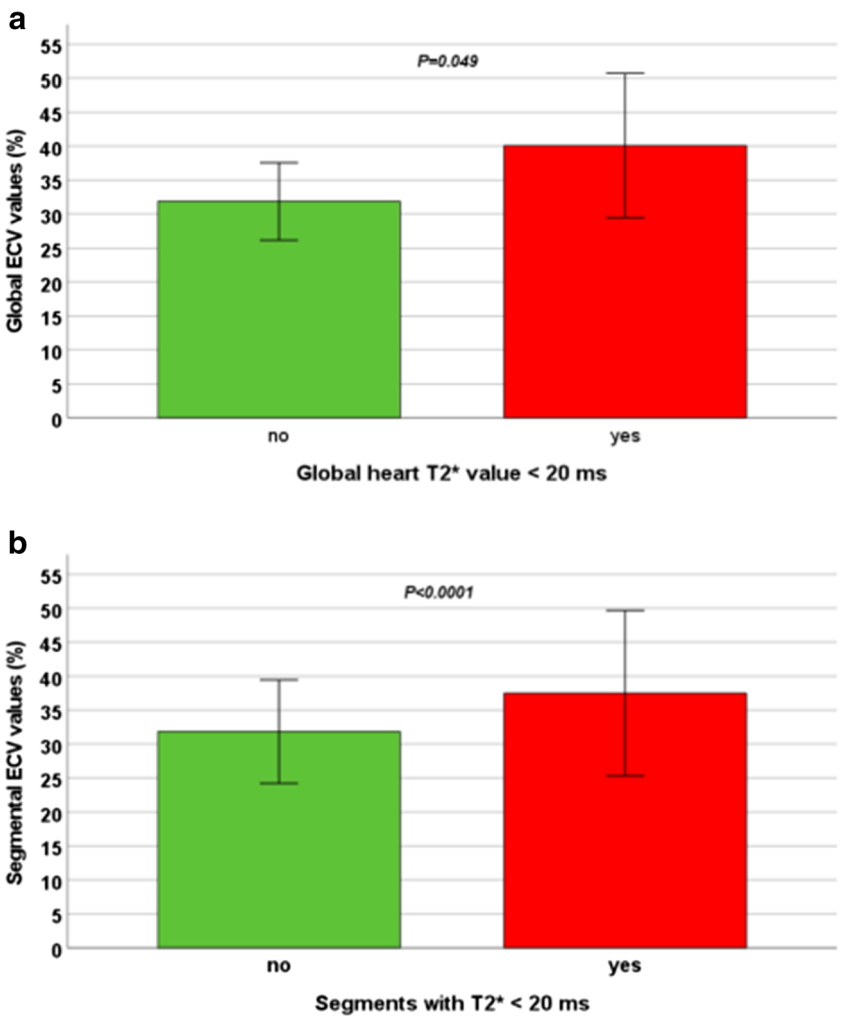

Fig. 4 **a** Global ECV values in TM patients without and with significant myocardial iron overload detected by the T2* technique. **b** Segmental ECV values in TM patients with normal and pathological segmental T2* values

(40.25 ± 9.74% vs. 32.63 ± 5.37%; p = 0.002) global heart T2 value.

Global ECV values were not correlated with LV end-diastolic volume index (R = 0.011; p = 0.911), mass index (R = −0.165; p = 0.088), or ejection fraction (R = 0.072; p = 0.461).

Global ECV was comparable between patients with and without replacement myocardial fibrosis (32.26 ± 5.93% vs. 33.95 ± 7.50%; p = 0.613). Segments with LGE (N = 126) had comparable ECV values than LGE-negative segments (32.33 ± 8.26% vs. 32.37 ± 8.65%; p = 0.947). Patients with replacement myocardial fibrosis had significantly lower global heart T1 values (924.56 ± 99.86 ms vs. 976.35 ± 74.71 ms; p = 0.012) and T2* values (34.66 ± 11.57 ms vs. 39.48 ± 7.26 ms; p = 0.045).

ECV and cardiac complications

Seventeen (15.7%) patients had a history of cardiac complications: 10 HF, 6 arrhythmias (4 supraventricular and 2 ventricular), and one myocarditis.

Patients with cardiac complications had significantly lower global heart T1 values (914.11 ± 114.64 ms vs. 962.93 ± 82.05 ms; p = 0.047) but comparable T2* values (34.34 ± 12.33 ms vs. 38.11 ± 8.84 ms; p = 0.238) and T2 values (54.49 ± 6.76 ms vs. 56.12 ± 4.92 ms; p = 0.685). Global ECV values tended to be higher in patients with cardiac complications than in patients free of complications but the difference was not significant (34.98 ± 8.41 ms vs. 32.09 ± 6.14 ms; p = 0.096).

Compared to patients without HF, patients with a history of HF showed significantly lower global heart T1 values (882.68 ± 125.46 ms vs. 962.66 ± 81.90 ms; p = 0.047) and significantly higher global heart ECV values (36.63 ± 9.41 ms vs. 32.12 ± 6.14 ms; p = 0.039) (Fig. 5).

Discussion

Our study demonstrated several important findings regarding the characteristics and the clinical impact of myocardial ECV in well-treated and well-chelated TM patients. We used a segment-based analysis, which showed a good intra- and inter-operator reproducibility for segmental and global ECV values.

First, regardless of the LGE positivity, we found increased ECV in patients with TM compared to healthy subjects, suggesting the presence of DIF. This datum strongly reinforces the importance of exploiting the multi-parametric potential of CMR to detect and better characterize the myocardial involvement of TM patients.

We investigated the demographic and clinical correlates of myocardial ECV values in TM patients and we detected a gender difference, with females having higher ECV values. This finding may simply reflect normal physiological differences, in particular the larger myocardial interstitium and the higher capillary density in females [33]. As demonstrated in a smaller cohort of TM patients [13] and in patients with sickle cell anemia [34], ECV values resulted significantly associated to NTproBNP levels, a proven diagnostic biomarker for HF due to systolic dysfunction [35]. As in other patient populations [36], ECV did not correlate with conventional cardiovascular risk factors.

This is the first study evaluating the association between ECV values and all three relaxation times, decreased by the myocardial iron deposition causing microscopic magnetic field inhomogeneities [37]. The T2* technique is the method of choice for MIO assessment [2], being fast, reproducible [15], validated against human tissue [14, 38], and conveying prognostic information [4, 5]. We found a significantly increased myocardial ECV in TM patients with reduced global heart T2* value, suggesting that in TM MIO can play a pathophysiological role in the development of DIF. This hypothesis is

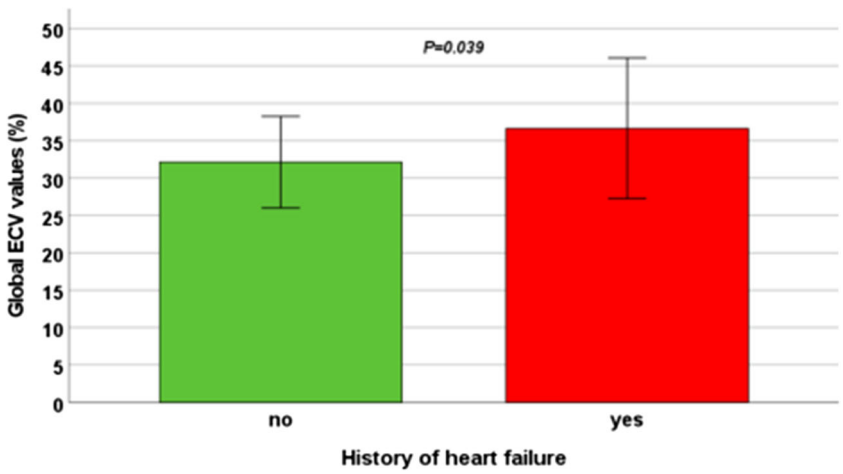

Fig. 5 Global ECV values in TM patients without and with a history of heart failure

supported by the fact that patients with reduced global heart T2 showed significantly higher global ECV values than patients with normal or increased global heart T2. As TM patients can develop chronic inflammation [39], the increased myocardial T2 value can be due to myocardial inflammation [37] that counterbalances the iron effects in non-heavily iron-overloaded hearts. Although myocardial inflammation can affect ECV, confounding the correlation between ECV and fibrosis [40], the absence of a difference in ECV values between patients with normal and increased global heart T2 value seems to suggest the dominance of fibrosis over inflammation. However, it is challenging to distinguish between myocardial inflammation and DIF based on imaging alone and a contribution of myocardial inflammation to the ECV increase cannot be completely ruled out, as also indicated by the presence of increased ECV in both patients with an active HCV infection. The absence of a link between decreased T1 and increased ECV may be explained by the fact that although in borderline patients T1 mapping has a higher sensitivity for MIO detection in comparison to the T2* technique [19, 41], T1 values are not specific for iron and show prolongation in presence of diffuse myocardial fibrosis [37]. The link between MIO and myocardial ECV is supported also by the negative correlation between MRI LIC and global heart T2*, T1, and T2 values and the positive correlation between MRI LIC and ECV values. The relationship between cardiac iron and liver iron is complex, due to the differences in iron uptake and elimination between the two organs, and the different efficacy of the available regimes on improving cardiac and hepatic iron load [42, 43]. So, controversial data are available in the literature regarding the cross-sectional correlation between cardiac and hepatic iron levels [28, 44–47]. Anyway, it is likely that the failure to control liver iron over the long term increases the risk of severe MIO [48].

No correlation was detected between ECV values and LV function, volumes, or mass, probably because the majority of our patients had normal or mild abnormal values of these parameters. Moreover, the ventricular size is strongly affected by the chronic anemia [26]. The lack of a correlation between myocardial ECV and LGE indicates that the increase in ECV was not driven by the presence of LGE and that focal and diffuse fibrosis may result from different underlying pathological processes. LGE is an all-or-nothing approach, allowing the detection of focal regions of replacement fibrosis or edema in an acute setting, but not of diffuse interstitial fibrosis [49].

Finally, we showed for the first time an association between increased ECV and history of HF. Interestingly, in our population of well-treated patients, generally not heavily loaded at the cardiac level because abnormal cardiac T2* prompted changes in clinical management, native T1 and ECV values turned out to be a more sensitive marker of HF, stronger than global heart T2* values. Increased ECV was not associated with the history of cardiac complications globally considered, likely because also supraventricular arrhythmias were considered and MIO was shown to contribute less to the development of supraventricular arrhythmias than to HF [4, 5].

Limitations

No histological test was performed to confirm the relationship between increased ECV and diffuse myocardial fibrosis in patients with thalassemia. However, due to its invasiveness, endomyocardial biopsy could not be justified.

This is a single-center study. Larger multicenter studies are needed to verify our findings and to test the transferability of ECV mapping.

Conclusions

CMR-derived myocardial ECV is increased in TM patients, irrespective of the presence of LGE. Increased myocardial ECV, potentially reflecting diffuse interstitial fibrosis, is significantly associated with MIO and with a history of heart failure. So, when possible, the ECV assessment should be included in the routine CMR of TM patients.

Longitudinal prospective studies are needed to clarify the temporal association between myocardial iron overload and diffuse fibrosis and to obtain a better understanding of the implications of the presence of diffuse myocardial fibrosis. Moreover, the potential of treatments targeted at interstitial fibrosis to reduce the HF risk should be investigated, especially in those patients refractory to iron chelating therapy.

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Declarations

Guarantor The scientific guarantor of this publication is Filippo Cademartiri.

Conflict of interest The authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

Statistics and biometry One of the authors has significant statistical expertise.

Informed consent Written informed consent was obtained from all subjects (patients) in this study.

Ethical approval Institutional Review Board approval was obtained.

Study subjects or cohorts overlap Some study subjects or cohorts have been previously reported in: Meloni A, Martini N, Positano V, et al. Journal of Cardiovascular Magnetic Resonance; 2021;23(1):70.

Methodology
- prospective
- cross sectional study/observational
- performed at one institution

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