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Original Article

Myocardial longitudinal strain as the first herald of cardiac impairment in very early iron overload state: an echocardiography and biosusceptometry study on beta-thalassemia patients

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Abstract: Background: Heart diseases due to iron overload are still the main cause of mortality in patients affected by beta-thalassemia. Detection of cardiac iron overload in pre-clinical stage allows tailoring of chelation therapy and follow-up strategies. Echocardiographic longitudinal strain analysis may be a useful tool for early detection of cardiac functional impairment iron-related. Methods: We examined 58 patients with beta-thalassemia on regular blood transfusion and iron chelation, without overt cardiac disease who had recent Biosusceptometry SQUID to quantify liver iron concentration and cardiac assessment by CMR T2*. Results: Average global longitudinal strain (GLS) was able to identify abnormal (<20 ms) cardiac T2* values with 96% specificity and negative predictive value of 92% (AUC 0.84, P=0.01). Apical 4-ch GLS may help identify early longitudinal impairment associated with severe liver iron overload with 96% specificity and negative predictive value of 92% (AUC 0.84, P=0.02). Patients with severe liver iron overload had lower average Global Longitudinal Strain values compared to other patients (*P*-value =0.005). Conclusion: GLS was a sensitive marker to detect both myocardial and liver iron overload in a population that is still free from cardiac symptoms. Thus, strain echocardiography may be a useful tool for early detection of iron overload in Beta-thalassemia.

Keywords: Thalassemia, echocardiography, strain, biosusceptometry SQUID

Introduction

In the last decades the survival of patients affected by thalassemia celebrated a progressive increase. This is to be attributed to both the improvement of scientific knowledge and to the introduction of new therapeutic strategies, above all iron chelation drugs [1].

Life expectancy and complication-free survival of these patients is still growing, modifying physicians' own view of thalassemia. Thalassemia was once considered a life-threatening, pediatric disease, now it has become a chronic disease that, if adequately treated, allows patients to live adult age [1, 2].

Nowadays, heart disease has dramatically declined as a cause of mortality in the thalassemic populations following standard therapy. Estimation of myocardial and hepatic iron overload by means of cardiac magnetic resonance (CMR) and Biosusceptometry SQUID (Superconducting Quantum Interference Device) have improved the ability to tailor chelation therapy to each single patient, thus reducing the rate of deaths from iron overload cardiomyopathy from 60% to 28% of deaths between 2010 and 2015 [3]. Cardiac monitoring earned a key role in the long-term management of these patients: the gold standard technique for detection of cardiac iron overload is still CMR with T2* measurement [4, 5]; however, echocardiography also have a relevant role in its monitoring [6].

Strain imaging is a relatively new tool in echocardiography that has been proven to be helpful in many different clinical conditions [7-10].

Indeed, myocardial strain-similarly to Tissue Doppler analysis - is the expression of ventricular wall deformation (meaning good contractile capacity) during the cardiac cycle [11].

Very few previous studies have directly investigated the potential role of strain imaging in iron-loading conditions [12]. In the present paper we tested whether myocardial strain parameters were able to stratify iron-loaded patients with particular attention to hepatic iron storage assessed with the gold standard Biosusceptometry SQUID (Superconducting Quantum Interference Device).

Since early detection of cardiac iron overload benefits the most those patients who do not present clinically detectable cardiac function impairment, we selected a sample of patients with transfusion-dependent beta-thalassemia and without overt signs of cardiac disease (see below the exclusion criteria).

Materials and methods

We enrolled consecutive transfusion-dependent thalassemia patients undergoing regular blood transfusion and iron chelation at our centers ("Reference Centre for Haemoglobinopathies, at San Luigi Hospital, Orbassano and "Blood Bank" at Molinette Hospital, Turin, Italy). The study was conducted in accordance with the Declaration of Helsinki. Although this study involves a prospective evaluation no ethical approval was obtained because it involved routine not-invasive procedures done for direct benefit of the patients: all included patients gave their informed consent on admission for data collection and future publications in anonymous studies.

Inclusion criteria

Patients with previous Biosusceptometry SQUID (Superconducting Quantum Interference Device) tand cardiac and liver MRI with T2* within the last 12 months.

Exclusion criteria

Clinical signs of heart failure, valvular heart disease, pulmonary hypertension, arrhythmias, left ventricular ejection fraction (LVEF) <55%, ongoing pregnancy, age <18 years or history of coronary artery disease, a poor-quality acoustic window non-suitable for strain measurement.

Transthoracic echocardiography was performed 4 to 9 days after blood transfusion to ensure hemodynamic stability, using a Vivid 7 GE (General Electric, USA) ultrasound system (2.5-3.5 MHz). The assessment was run by two expert physicians that were blinded to patients' clinical information until the end of the study. Cardiac cycles were stored in a digital format (Echopach, General Electric, USA). Chamber dimensions, flow Doppler and TDI were determined by standard procedures [13, 14]. Global longitudinal strain (GLS) was calculated by 2D specked tracking echocardiography (STE). For all the measurements, intervals from 3 consecutive cardiac cycles were stored and averaged.

Clinical and hematological data of the enrolled patients were collected. Liver iron concentration (LIC) was measured by SQUID biomagnetic susceptometer (Model 5700 Tr istan Technology, S.Diego CA). In order to distinguish among different degrees of liver iron overload we chose the universally-accepted cut-off of 7 mg/g dry weight (corresponding to 1200 mcg/g wet weight for liver iron concentration by SQUID) to define a moderate iron concentration and a LIC>15 mg/g d. w. (corresponding to 2571 mcg/g w.w.) for severe iron liver concentration [15].

We defined moderate cardiac iron overload if T2*<20 ms and severe cardiac iron overload if T2*<10 ms, according to literature [16].

Since our main working hypothesis was myocardial strain reduction as an early sign of iron toxicity, we divided our population in two subgroups according to strain values. On the basis of the literature [12, 17-20], we selected two possible cut-off values of GLS, specifically -19% and -17.5%, to define abnormal myocardial strain.

Statistical analysis

All data were analyzed using SPSS version 20.0 (IBM) statistical package. Categorical data are presented as frequency and percentage (%). Continuous variables were presented as mean ± standard deviation (SD), median, minimum and maximums. The comparisons among groups were made by unpaired Student t test or Fisher's exact test for continuous variables with normal distribution. Mann-Whitney U tests was used for continuous variables with non-normal

Table 1. Clinical characteristics of the study population (N=58)

	median	First quartile	third quartile	min	max
Start of Chelation (age in years)	3.85	2.8	6.275	1	33.9
First Transfusion (age in years)	0.8	0.4	1.3	0.2	33.4
Serum Ferritin μg/L (mean)	778.85	396	2162	155	6891
LIC (last)	1308	1079	2011	664	5053
AST (mean)	32.29	26.58	40.8	18.96	130.42
ALT (mean)	32.89	19.1	62.3	10.25	199.52
T2* heart (last)	39.705	33.27	45.68	7.37	56.42
T2* liver (last)	9.86	4.79	15.06	0.68	37.19
Stiffness (Fibroscan)	5.13	4.43	6.83	3.5	12.4
Serum Ferritin μg/L (mean)	778.85	396	2162	155	6891
LIC (last)	1308	1079	2011	664	5053
T2* heart (last)	39.705	33.27	45.68	7.37	56.42
T2* liver (last)	9.86	4.79	15.06	0.68	37.19
Age at diagnosis (years)	0.6	0.375	1	0.1	3.8

AST = aspartate transaminase; ALT = alanine transaminase; LIC = liver iron concentration.

Table 2. Echocardiographic characteristics (N=58)

Echocardiographic Findings	T2*<20 ms	T2*>20 ms	*P-value*	LIC>2751 mcg/g	LIC<2751 mcg/g	*P-value*
LV EF (%)	59±2	63±6	0.02	59±5	64±5	0.19
LV EDD (mm)	47±2	48±5	0.35	47±3	48±5	0.08
LV ESD (mm)	26±6	29±3	0.03	28±3	29±4	0.28
Indexed LA Volume (ml)	51±3	46±14	0.03	47±25	49±18	0.43
Mitral E wave (m/s)	0.74±0.1	0.89±0.2	0.22	0.86±26	0.87±15	0.04
Mitral A wave (m/s)	0.49±0.1	0.61±0.2	0.17	0.59±20	0.60±16	0.49
Mitral E/A	1.5±0.3	1.6±0.6	0.24	1.7±0.9	1.6±0.6	0.09
Lateral systolic Velocity (mm/sec)	10.7±2.8	10.7±2.6	0.91	9±3	11±2	0.57
Lateral early diastolic Velocity (mm/sec)	14.5±1.5	15.7±4.1	0.04	14±3	16±4	0.63
RV EDD (mm)	33±1	34±4	0.04	33±2	34±3	0.03
GLS AVG (%)	19.6±2.6	20.4±3.7	0.98	18.7±3.3	20.6±3.6	0.45
GLS 4CH (%)	18.6±3	20.4±2.6	0.91	17.3±2.4	20.7±2.4	0.79
PAPs (mmHg)	25±5	24±5	0.71	25±4	25±6	0.10

LV = left ventricle; RV = right ventricle; EF = ejection fraction; EDD = end-diastolic diameter; ESD = end-systolic diameter; LA = Left Atrium; PAPs = pulmonary artery pressure; GLS AVG = average Global Longitudinal Strain; GLS 4CH = four-chamber apical view GLS.

distribution and a Chi-squared test for categorical variables. Pearson test was used to evaluate correlations. Receiver-operating characteristic (ROC) curve analysis was done to find a cut off point for GLS based on detection of cardiac iron overload. A two-tailed *P* value <0.05 was considered statistically significant.

Results

From November 2017 to March 2018 we enrolled 64 consecutive patients, of whom 58 had an acoustic window suitable for strain measurement and were finally included in the analysis. Clinical and echocardiographic characteristics are reported in **Tables 1** and **2**. Of note, 29 patients were females (50%) and 29 were males (50%). The mean age was 40.4 years±7.9 years old (range 21-59). Fifty-four patients had a diagnosis of Thalassemia major, 3 had Thalassemia intermedia and 1 Sickle-beta thalassemia, and 65.5% of population was splenectomized. The median value of average global myocardial longitudinal strain in our

Table 3. Myocardial strain (in percentage) in studied population (N=58)

	Mean ± SD	Median	IQ Range	Range
GLS_LAX	-18.84±3.79	-20.40	-(18.30-22.40)	-(11.50-30.40)
GLS_A4C	-19.59±2.69	-19.80	-(18.60-22.30)	-(14.40-26.60)
GLS_A2C	-20.87±3.16	-21.30	-(19.30-23.40)	-(13.50-27.10)
GLS_Avg	-18.41±3.57	-21.00	-(19.70-22.20)	-(13.00-24.30)

GLS = Global Longitudinal Strain; LAX = long axis view; A4C = apical 4-chambers view; A2C = apical 2-chambers view; GLS_Avg = Average global longitudinal strain; IQ = inter quartile.

Table 4. Different distribution of main overload parameters according to average GLS cut-off

	GLS Avg<17.5 (n=5)	GLS Avg>17.5 (n=53)	*P-value*	GLS Avg<19.5 (n=12)	GLS Avg>19.5 (n=46)	*P-value*
EF (%)	56	64	0.01	59	64	0.01
T2* Heart (ms)	22	39	0.002	33.2	38.9	0.14
LIC (mcg/g)	3429	1477	0.001	2272	1482	0.02
Stiffness	6.5	5.8	0.47	6.6	5.7	0.12
T2* Liver (ms)	7.7	11.4	0.29	11	11	1.0

GLS Avg = Average global longitudinal strain; EF = ejection fraction; LIC = liver iron concentration.

sample was -20.40% (IQ range 18.30-22.40 %), ranging from 11.50% to 30.50% (**Table 3**).

Cardiac iron overload

A total of 50 patients had T2*>20 ms (average T2* of 41 ms; range 22-56), while eight patients had pathological T2* values (i.e. <20 ms, with average T2* of 12 ms; range 7-18).

A ROC curve exploration was performed and a GLS cut-off value corresponding to -17.5% was showed to have a 50% sensitivity and 96% specificity to detect abnormal T2* values (AUC 0.846; P=0.011; see **Table 4** and **Figure 2**) with a negative predictive value of 92% and a positive predictive value of 67%.

Hepatic iron overload

We evaluated Liver Iron Concentration assessed by SQUID. The correlation between the GLS Avg levels with the Liver Iron Concentration (LIC) was significant (r=-0.687, P=0.001). A cut-off of 15 mg/g dry weight [21] was established in order to detect patients with severe hepatic iron overload. The two groups had significantly different prevalence of pathological GLS: patients with liver iron concentration <15 mg/g d.w. had abnormal GLS (i.e. <19.0) in 14.2% of cases while patients with severe liver iron overload had abnormal average GLS in 55.6% of cases, with a *P*-value of 0.005. Again, the best cut-off resulted an average GLS<17.5, which showed a Sensitivity of 50% and Specificity of 83%, with a negative predictive value of 88% and a positive predictive value of 83% (**Figure 1**). Interestingly, patients with severe hepatic iron overload showed worse myocardial load too (mean T2* 21 ms *vs* 40 ms). The relationship among heart iron concentration, liver iron concentration and strain values is reported in **Figure 3**.

Discussion

The present study investigated the effectiveness of myocardial strain measurement for the early detection of iron toxicity in patients with thalassemia.

We analyzed a sample of 58 patients undergoing the gold standard treatment for beta-thalassemia, that is regular transfusion and intensive iron chelation therapy. The median value of average global myocardial longitudinal strain in our sample was -20.40%: Pizzino et al. [22] reported a similar median value (-20.6%) in a group of 28 patients with similar characteristics, as well as Abtahi et al. [10] who found a mean value of 19.36%.

We screened and assessed *cardiac iron overload* by means of CMR T2* values, which is

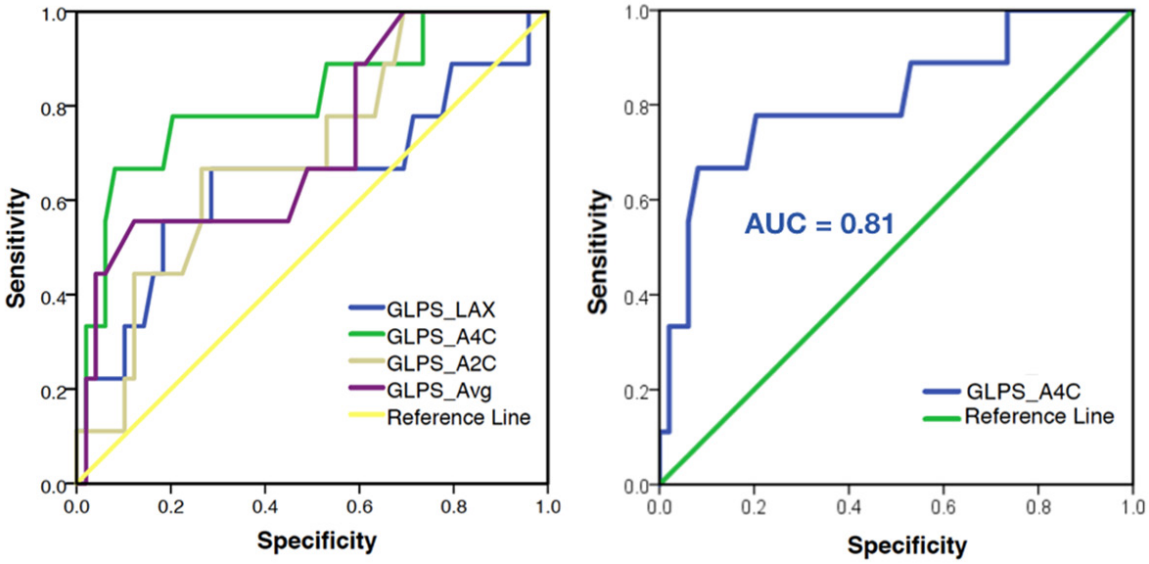

Figure 1. Diagnostic power of Strain Imaging parameters for detection of severe liver iron overload (LIC>2571 mcg/g w.w) in beta-thalassemia patients described by ROC curves. Area under curve values are respectively: 0.65 for apical Long axis Global Longitudinal Strain (GLS); 0.81 for apical 4 chamber GLS; 0.70 for apical 2 chamber GLS; 0.72 for average GLS. N=58 (Severe overload N=9). On the right side isolated ROC curve for apical 4 chamber GLS performance.

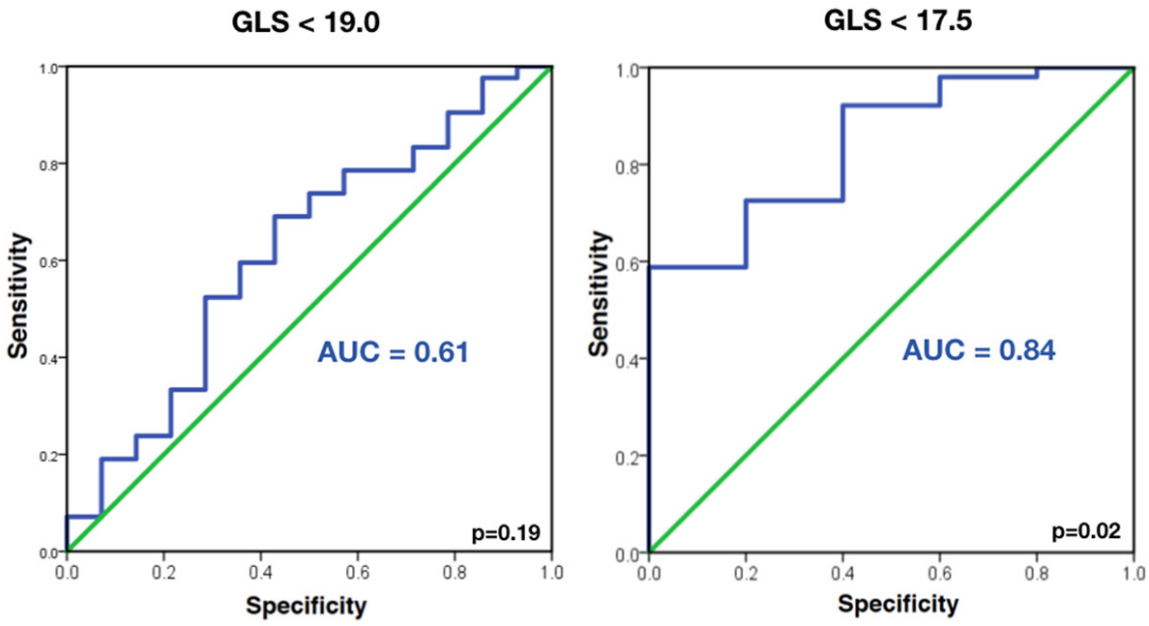

Figure 2. Different diagnostic power of Strain Imaging parameters (GLS cut-off -19.0 and -17.5%) for detection of cardiac iron overload (T2*<20 ms) in beta-thalassemia patients described by ROC curve (N=58).

the gold standard for iron overload assessment in these patients [16]. Previous studies reported a positive association between myocardial strain and T2* reduction [20, 22-24], and in our population a cut-off of 20 ms [5] was used to test the ability of echocardiographic global longitudinal strain to effectively screen patients: the ROC Curve analysis showed that a GLS cut-off of 17.5% was able to rule-out abnormal T2* values (i.e <20 ms) with a 92% negative predictive value (**Figure 2**).

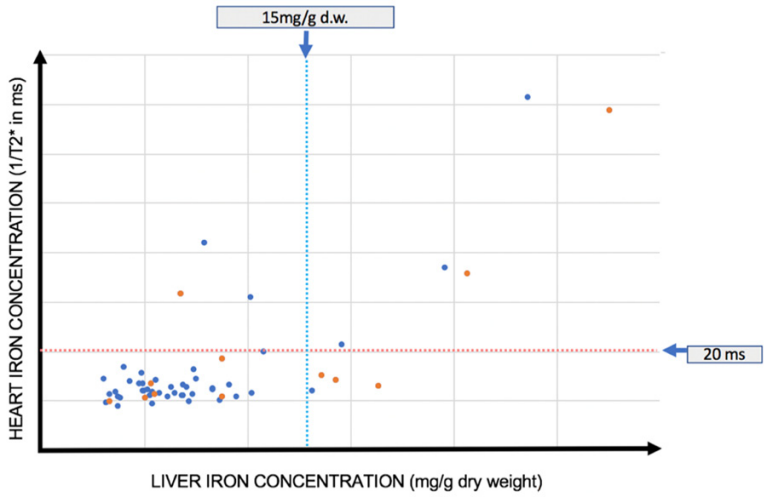

Figure 3. Scatterplot reporting heart and liver iron concentration of studied population (N=58). Blue points correspond to patients with normal strain values. Orange points correspond to patients with pathological myocardial strain values.

Several studies have previously evaluated the correlation of various echocardiographic parameters with cardiac MRI in thalassemic patients [20, 22-24]. Pizzino et al., for example, reported average GLS to be significantly lower (*P*-value <0.001) in patients with T2*<20 ms in a population similar to ours as regards age (median 37.4 years), clinical history and treatment. Of note, 21.4% of patients in Pizzino et al.'s study had T2*<20 ms whereas the sample in our study consisted of cardiac complication-free population in which only 12.1% of the patients had T2*<20 ms, this justifying the different GS cut-off performance in our population.

A positive association between myocardial strain and T2* was also shown by Ari et al. [23] and by Gupta et al. [24]. However, these studies focused on pediatric patients (median age 13.2 and 12.5 years old, respectively), therefore, results cannot be directly compared to those of the present study.

In the study by Parsae et al. [20], a significant correlation between T2* and global longitudinal strain was also found (*P*-value <0.001), but again the population involved in the study had a very high rate of abnormal (i.e. <20 ms) T2* values (31.3%).

The recent study published by Abtahi et al. [12] was very similar to the present one in terms of study design. Their results, similarly to ours, showed the efficacy of GLS for the detection of cardiac iron overload and described this diagnostic power by means of ROC curves. But the study by Abtahi and colleagues included a much younger population (mean =27 years old) compared to ours (mean =40 years old). In fact, the higher specificity of GLS they find for detection of T2* overload (86.6% vs 60%) could be attributed to the younger age of the studied population, in which the iron overload is the only reasonable cause of deflection of contractile function; actually, they reported a mean CMR T2* of 19.76±10.29 ms, a value someway worse than our, and indicating a population with higher cardiac overload.

What we really think our study could add as new insights into the screening and early recognition of iron overload status in thalassemic patients, is the presence of correlation of pathological GLS value with very early iron overload, not yet in the heart but in the liver. As regards *hepatic iron overload*, Liver Iron Concentration assessed by SQUID proved to be a strong tool for its measurement [21]. As far as we know, no correlation between hepatic iron overload and myocardial strain impairment had been proved so far in a similar population as we did. We showed that a GLS average value <17.5 is able to predict liver iron overload with a 83% positive predictive value and a 88% negative predictive value. This correlation is extremely interesting because we know that iron accumulates early in the liver and only after some time into the heart. An increase in hepatic storage without evidence of heart loading at CMR therefore depicts a population in which the iron cardiomyopathy is in a very early stage. If higher hepatic iron levels are so strongly correlated with longitudinal strain impairment, we could speculate that iron may be able to affect early longitudinal contraction even before an actual deposit into the heart fibers is detectable by CMR. This could be the reason why the apical four-chamber GLS measurements appear to be the more sensitive strain parameter in our

study. Furthermore, it has been already showed that it could add as an effective surrogate of average GLS in absence of regional wall motion abnormalities [22]. Since the gold standard test for Liver Iron Concentration is the liver T2* which is expensive and not always available, and since SQUID technology has strong diagnostic power but even more scarce availability (just three of them in the world), our study suggests that the assessment of GLS can be used as a useful, more available and cheaper tool for both the screening of hepatic iron load, helping prevent myocardial diseases like heart failure and arrhythmias [25].

In other words, Global longitudinal strain was an extremely sensitive marker to highlight a stage preceding myocardial iron overload. Actually, in our relatively old thalassemic population mainly with mild signs of iron overload, a cut-off of -19% global longitudinal strain was able to detect severe liver iron overload, while the -17.5% cut-off was able to discriminate cardiac iron overload. The low specificity of average GLS may reflect the effects of the early stage of the disease, while apical four chamber GLS showed the best correlation, probably due to early impairment of longitudinal contraction.

Limits

The sample size of our study (n=58) is relatively small, however, previously published reports on related topics have relied on similar numbers [12, 22-24]. Secondly, we did not include a control group in our study, limiting our findings to the analysis of a thalassemia-affected population. In addition, the design of our study was cross-sectional and we could not evaluate the sensitivity of GLS for early detection of cardiac iron overload during long term monitoring and follow up. Indeed, repeated GLS measurements across time could be useful to evaluate the hypothesis of GLS as an "intermediate station" of iron toxicity between liver and heart overload. Although the design of our study would have greatly benefitted from performing echocardiography, cardiac MRI and SQUID within the same day, this has not been possible due to logistic reasons such as time constrictions, machines availability and location. Consequently, we had to rely on pre-existing assessments of cardiac MRI and SQUID, which anyway, were less than a year older.

Conclusion

Speckle-Tracking Echocardiography assessing 2-D strain imaging is an experimental technique that has shown a good diagnostic potential in several fields, including iron overload monitoring.

In our study, Global longitudinal strain was a sensitive marker of early iron overload, with an interesting association between abnormal longitudinal strain values and severe hepatic iron overload which is known to precede cardiac overload. This finding suggests that the assessment of GLS can be a useful and cheaper tool for screening of myocardial and hepatic iron overload, to better select patients to investigate with CMR and SQUID (more expensive and less available).

Disclosure of conflict of interest

None.

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References

[1] Longo F, Corrieri P, Origa R, Barella S, Sanna PMG, Bitti PP, Zuccarelli A, Commendatore FV, Vitucci A, Quarta A, Lisi R, Cappellini MD, Massei F, Forni GL and Piga A. Changing patterns of thalassaemia in Italy: a WebThal perspective. Blood Transfus 2021; 19: 261-283.

[2] Farmakis D, Giakoumis A, Angastiniotis M and Eleftheriou A. The changing epidemiology of the ageing thalassaemia populations: a position statement of the Thalassaemia International Federation. Eur J Haematol 2020; 105: 16-23.

[3] Voskaridou E, Kattamis A, Fragodimitri C, Kourakli A, Chalkia P, Diamantidis M, Vlachaki E, Drosou M, Lafioniatis S, Maragkos K, Petropoulou F, Eftihiadis E, Economou M, Klironomos E, Koutsouka F, Nestora K, Tzoumari I, Papageorgiou O, Basileiadi A, Lafiatis I, Dimitriadou E, Kalpaka A, Kalkana C, Xanthopoulidis G, Adamopoulos I, Kaiafas P, Mpitzioni A, Goula A, Kontonis I, Alepi C, Anastasiadis A, Papadopoulou M, Maili P, Dionisopoulou D, Tsirka A, Makis A, Kostaridou S, Politou M and Papassotiriou I; Greek Haemoglobinopathies Study Group. National registry of hemoglobinopathies in Greece: updated demographics, current trends in affect-

ed births, and causes of mortality. Ann Hematol 2019; 98: 55-66.
[4] Kirk P, Roughton M, Porter JB, Walker JM, Tanner MA, Patel J, Wu D, Taylor J, Westwood MA, Anderson LJ and Pennell DJ. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. Circulation 2009; 120: 1961-8.
[5] Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, Firmin DN, Wonke B, Porter J, Walker JM and Pennell DJ. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001; 22: 2171-9.
[6] Barbero U, Longo F, Destefanis P, Gaglioti CM, Pozzi R and Piga A. Worsening of myocardial performance index in beta-thalassemia patients despite permanently normal iron load at MRI: a simple and cheap index reflecting cardiovascular involvement? IJC Metab Endocr 2016; 13: 41-44.
[7] Haugaa KH, Goebel B, Dahlslett T, Meyer K, Jung C, Lauten A, Figulla HR, Poerner TC and Edvardsen T. Risk assessment of ventricular arrhythmias in patients with nonischemic dilated cardiomyopathy by strain echocardiography. J Am Soc Echocardiogr 2012; 25: 667-673.
[8] Weidemann F, Mertens L, Gewillig M and Sutherland GR. Quantitation of localized abnormal deformation in asymmetric nonobstructive hypertrophic cardiomyopathy: a velocity, strain rate, and strain Doppler myocardial imaging study. Pediatr Cardiol 2001; 22: 534-537.
[9] Fadel B, Baldini L, Pergola V, Al Bulbul Z and Di Salvo G. Myocardial deformation imaging and rare cardiomyopathies with hypertrophic phenotype: a review focused on Fabry disease, Friedreich ataxia and amyloidosis. Cardiogenetics 2013; 3: 4.
[10] Bussadori C, Di Salvo G, Pluchinotta FR, Piazza L, Gaio G, Russo MG and Carminati M. Evaluation of right ventricular function in adults with congenital heart defects. Echocardiography 2015; 32 Suppl 1: S38-52.
[11] Barbero U, Destefanis P, Pozzi R, Longo F and Piga A. Exercise stress echocardiography with Tissue Doppler Imaging (TDI) detects early systolic dysfunction in beta-thalassemia major patients without cardiac iron overload. Mediterr J Hematol Infect Dis 2012; 4: e2012037.
[12] Abtahi F, Abdi A, Jamshidi S, Karimi M, Babaei-Beigi MA and Attar A. Global longitudinal strain as an indicator of cardiac iron overload in thalassemia patients. Cardiovasc Ultrasound 2019; 17: 24.
[13] Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS and Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440-63.
[14] Nagueh SF, Appleton CP, Gillebert TC, Marino PN, Oh JK, Smiseth OA, Waggoner AD, Flachskampf FA, Pellikka PA and Evangelisa A. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. Eur J Echocardiogr 2009; 10: 165-93.
[15] St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, Pootrakul P, Robins E and Lindeman R. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood 2005; 105: 855-61.
[16] Pennell DJ, Udelson JE, Arai AE, Bozkurt B, Cohen AR, Galanello R, Hoffman TM, Kiernan MS, Lerakis S, Piga A, Porter JB, Walker JM and Wood J; American Heart Association Committee on Heart Failure and Transplantation of the Council on Clinical Cardiology and Council on Cardiovascular Radiology and Imaging. Cardiovascular function and treatment in β-thalassemia major: a consensus statement from the American Heart Association. Circulation 2013; 128: 281-308.
[17] Dandel M, Lehmkuhl H, Knosalla C, Suramelashvili N and Hetzer R. Strain and strain rate imaging by echocardiography-basic concepts and clinical applicability. Curr Cardiol Rev 2009; 5: 133-148.
[18] Sutherland GR, Di Salvo G, Claus P, D'hooge J and Bijnens B. Strain and strain rate imaging: a new clinical approach to quantifying regional myocardial function. J Am Soc Echocardiogr 2004; 17: 788-802.
[19] Di Salvo G, D'Aiello AF, Castaldi B, Fadel B, Limongelli G, D'Andrea A, Pergola V, Pacileo G, Del Giudice EM, Perrone L, Calabrò R and Russo MG. Early left ventricular abnormalities in children with heterozygous familial hypercholesterolemia. J Am Soc Echocardiogr 2012; 25: 1075-82.
[20] Parsaee M, Akiash N, Azarkeivan A, Alizadeh Sani Z, Amin A, Pazoki M, Samiei N, Jalili MA, Adel MH and Rezaian N. The correlation between cardiac magnetic resonance T2* and left ventricular global longitudinal strain in people with β-thalassemia. Echocardiography 2018; 35: 438-444.

[21] Wunderlich AP, Cario H, Juchems MS, Beer M and Schmidt SA. Noninvasive MRI-based liver iron quantification: methodic approaches, practical applicability and significance. Rofo 2016; 188: 1031-1036.

[22] Pizzino F, Meloni A, Terrizzi A, Casini T, Spasiano A, Cosmi C, Allò M, Zito C, Carerj S, Aquaro GD, Di Bella G and Pepe A. Detection of myocardial iron overload by two-dimensional speckle tracking in patients with beta-thalassaemia major: a combined echocardiographic and T2* segmental CMR study. Int J Cardiovasc Imaging 2018; 34: 263-271.

[23] Ari ME, Ekici F, Çetin İİ, Tavil EB, Yaralı N, Işık P, Hazırolan T and Tunç B. Assessment of left ventricular functions and myocardial iron load with tissue Doppler and speckle tracking echocardiography and T2* MRI in patients with β-thalassemia major. Echocardiography 2017; 34: 383-389.

[24] Gupta A, Kapoor A, Phadke S, Sinha A, Kashyap S, Khanna R, Kumar S, Garg N, Tewari S and Goel P. Use of strain, strain rate, tissue velocity imaging, and endothelial function for early detection of cardiovascular involvement in patients with beta-thalassemia. Ann Pediatr Cardiol 2017; 10: 158-166.

[25] Barbero U, Fornari F, Guarguagli S, Gaglioti CM, Longo F, Doronzo B, Anselmino M and Piga A. Atrial fibrillation in β-thalassemia major patients: diagnosis, management and therapeutic options. Hemoglobin 2018; 42: 189-193.