No evidence of cardiac iron in 20 never- or minimally-transfused patients with thalassemia intermedia

Iron loading in non-transfused thalassemia intermedia (TI) patients is mainly due to an increased intestinal absorption secondary to chronic anemia and varies from 2 to 5 grams per year.[1] In thalassemia major (TM) iron derived from red blood cell breakdown accumulates first in the reticuloendothelium and later in parenchymal cells. By contrast in TI, as in genetic hemocromatosis (HH), parenchymal cells are the first and remain the most involved.[2] Therefore, patients with TI can develop a variety of iron overload related complications. In TI patients, liver iron concentration (LIC) disproportionate to the increased serum ferritin has been observed as a result of hepcidin deficiency secondary to the strong erythropoietic drive.[3]

Heart disease is still the main cause of death in TM and myocardial iron loading is detected by magnetic resonance (MRI) in about two thirds of patients maintained on chelation treatment with desferioxamine.[4]

Cardiac involvement in TI is mainly characterized by a high-output state and pulmonary hypertension, with systolic left ventricle function usually preserved.[5]

However, heart iron overload in patients with TI has still not been extensively studied. The aim of this study was to assess the prevalence of myocardial iron overload in a cohort of never or minimally transfused TI patients by means of the new MRI T2-star (T2*) technique, widely used in TM, and to evaluate its correlation with heart function, LIC and serum ferritin. The study was designed according to the standards of Good Clinical Practice and approved by the Hospital Ethics Committee. Informed consent was obtained from all participants.

Twenty adult patients with TI (mean age 35±11 years, range 18-54) under treatment at the Day Hospital of the Talassemia Età Evolutiva, Ospedale Regionale Microcitemie, Cagliari were evaluated. Main criteria for the sample selection were transfusion independence and lack of a regular iron chelation because of poor compliance or side effects. Thirteen of the examined patients had never been transfused and 7 had received only sporadic transfusions during infections or surgery (less than 10 blood units in total). Genotypes and other hematologic findings are summarized in Table 1. Ferritin and hemoglobin values are the mean of all measurements (at least 4) taken in the year before the MRI evaluation. All patients underwent heart MRI and, in a subgroup of 11 patients, it was also possible to obtain LIC through MRI. To measure myocardial iron, patients were scanned with a commercially available 1.5 T Magnet (GE Milwaukee, USA) with a torso PA coil using a multiecho breath hold sequence according to Westwood *et al.*[6] A T2* above 20 msec was considered normal. LIC was evaluated through the measurement and imaging of proton transverse relaxation rates (R2) within the liver, sent to a post-image processing service (Ferriscan®-Resonance Health, Australia).[7] LIC between 0.17 and 1.8 mg/g d.w. was considered within the normal range. Left ventricular function was assessed by echocardiography, using Hewlett Packard Agilent Sonos 5500 phase-array scanner (Agilent, Andover, MA, USA). In the absence of a reference range for TI, a left ventricular ejection fraction (LVEF) above 55%, which is the cut-off for normal individuals, was considered normal. Heart T2* was normal in all patients (mean value 43±6.3 msec; range 35-61 msec) and no correlation with hemoglobin or ferritin values was found (p=0.23 and 0.78, respectively) although this may have been influenced by the small size of the sample. Our finding is not completely consistent with other reports in which a subgroup of TI patients showed moderate cardiac iron overload.[8,9] This could be explained by the different age and number of transfusions received in the patients studied. LIC was within the normal range in 4 out of 11 patients. Mild to severe liver iron overload (mean LIC 8±9 mg/g d.w.; range 1.9-27.9) was found in the other 7 patients (63.6%). Although the number of patients was small, we found a significant correlation and a high predictive value of serum ferritin for LIC (p<0.001, Rs=0.77, Spearman's rank correlation coefficient). Larger groups should be assessed in the future to confirm this finding. Systolic function was normal in all but one examined patient (mean LVEF 62.6±6.5%, range 56-78.3%). The patient with the abnormal LVEF (50%) (Patient 9 in Table 1) was 51 years old and had a left ventricular dilatation.

This body iron distribution is consistent with the recent report by Gardenghi *et al.* in two mouse models of TI, which primarily accumulate iron in the liver and do not accumulate iron in the heart over time.[10] On the other hand, since in TI iron is mainly absorbed through the gut and first passes through the liver, it is reasonable that the liver would be loaded first. A comparison between TI and HH is not possible since cardiac MRI is not routinely used in this condition. However, in most cases of HH, congestive cardiac failure and arrythmias, which are the classical cardiac abnormalities possibly related to iron deposition in the myocardium and conducting system, occur in old age.[11]

Table 1. Genotype, hematologic findings, liver iron concentration (Ferriscan®) and heart MRI T2* in 20 patients with thalassemia intermedia.

	Age (years)	Genotype	Hb (g/dL)	Ferritin (ng/mL)	LIC (ng/g d.w.)	Heart T2* (msec)
1	26	β39/β39	9	650	6.9	44
2	30	β39/β39	8.2	811		50.4
3	34	β39/β39	9.2	422		44.3
4	18	β39/β39	9.5	465	4.3	50.5
5	24	β39/β39	9.2	540	8.2	61
6	59	β39/β39	8.4	211		41
7	24	β39/β39	9.5	246	1.7	38.3
8	51	β39/β39	9.6	304		36.1
9	51	β39/β39	8.8	339	1	39.3
10	43	β39/β39	8	3320	27.9	38
11	29	β39/β6(-A)	8.2	280		35
12	29	β39/β6(-A)	7.9	633	4.6	49.5
13	26	β39/δβ	13.3	318	1.4	50.1
14	33	β39/δβ	8.5	170	1.2	38.
15	43	β39/δβ	9.3	990		43
16	35	β39/βsilent	6.9	70		42
17	37	β39/βsilent	8.2	800		38
18	32	β39/βIVS1-6	6.9	265	2.2	41.9
19	28	β39/β-87	8.3	210	1.9	41.5
20	54	β^{-}/βααα/αα	9.9	119		43.7
mean±SD	**37.2±10**		**8.8±1.3**	**558±697**	**5.6±7.8**	**43±6.3**

In conclusion, in never or minimally transfused patients with thalassemia intermedia we found no evidence of cardiac iron overload, while there may be significant hepatic iron accumulation. However, since cardiac MRI has not been routinely used in TI, further studies and longer follow-up are needed to understand if and when detectable cardiac iron deposition can occur. Therefore, all patients with TI, and especially those who do receive occasional transfusions, should be evaluated regularly for cardiac and liver iron overload.

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Defective mRNA levels are responsible for a β-thalassemia phenotype associated with Hb Federico II, a novel hemoglobin variant [β-106 (G8) Leu→Val]

Hemoglobinopathies are widespread monogenic disorders that encompass complex and partially overlapping hemoglobin disorders and thalassemia syndromes. About 960 hemoglobin variants have been identified, some of which are reported to be unstable.[1] Various mechanisms for the decreased stability of a hemoglobin variant, which gives rise to differences in clinical manifestations, have been proposed. In the case of thalassemic hemoglobinopathies, structural changes are associated to quantitative defects of the corresponding globin chain, thus generating typical thalassemic phenotypes.[2]

Although it is generally agreed that clinical effects are related to an abnormal protein, it is conceivable that in some cases the globin variant may impair expression mechanisms by producing aberrant mRNA that could either be inadequately processed or be unstable possibly as a consequence of changes in the secondary structure. However, this intriguing hypothesis has yet to be demonstrated.[3]

As we previously reported,[4] during screening for couples at risk for β-thalassemia we had examined a patient presenting with hypochromic microcytic anemia and increased HbA2 level, not associated to any clinical alteration (Figure 1A). No abnormal hemoglobin fractions were observed at the cation-exchange HPLC analysis or cellulose acetate electrophoresis. Morphological analysis of red blood cells revealed anisopoikilocytosis with moderate microcytosis; erythrocytes showed increased osmotic resistance and absence of Heinz bodies. Thermal and isopropanol hemoglobin stability tests were negative although these data are not completely reliable due to the limited amount of circulating abnormal variant. Serum iron, transferrin, ferritin and bilirubin levels were normal.

Molecular analysis was performed on DNA and RNA from whole blood samples after local Ethics Committee approval and informed consent were obtained. β-globin gene sequence analysis was performed on PCR products encompassing the entire genomic sequence from 600 bp upstream from the initiation transcription site to 170 bp downstream from the termination codon, as previously reported.[5] Rearrangements in the α-globin gene cluster were excluded by Southern blotting.[6]

Sequence analysis revealed a mutation at heterozygous level in the third exon of the β-globin gene, which caused the substitution of the Leu residue with a Val residue (CTG→GTG) at codon 106, thereby producing a novel hemoglobin variant (Figure 1B). We designated this variant *Hb Federico II*.[4] No other sequence alterations were detected in the β-globin gene, which strongly suggests that this mutation is associated to a β-thalassemic trait. Hb Federico II was undetectable at cation-exchange HPLC analysis and produced a small abnormal peak (7-10% of total hemoglobin) at reverse-phase HPLC which eluted before the β-globin peak.