rate of inadequate biopsies. We also recommend that standardized evidence-based criteria are developed to determine what constitutes an adequate pediatric bone marrow biopsy to reduce interpathologist variability.

■ Author Contributions

R.C.G., F.S., M.A., S.W.A., and M.C. designed the research. R.C.G. collected and analyzed the data, and wrote the manuscript. R.C.G., F.S., M.A., S.W.A., and M.C. reviewed and edited the manuscript.

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Pancreatic iron overload by T2* MRI in a large cohort of well treated thalassemia major patients: Can it tell us heart iron distribution and function?

To the Editor: We evaluated pancreatic iron by magnetic resonance imaging (MRI) in a large cohort of well-treated thalassemia major (TM) patients and we assessed its relationship with myocardial iron overload (MIO) using a segmental approach and with cardiac function.

We studied 147 TM patients (70 males, 31.73 ± 8.12 years) enrolled in the MIOT (MIO in Thalassemia) Network [1].

The disturbances of glucose metabolism were assessed within three months from the MRI by means of oral glucose tolerance test (OGTT).

MRI was performed using a 1.5 T scanner. Iron overload (IO) was measured using a T2* gradient-echo multiecho sequence and the images were analyzed using a previously validated software (HIPPO-MIOT®). T2* values over pancreatic head, body and tail were assessed and global value was the mean [2]. The lowest threshold of normal T2* value was 26 ms [2]. Liver T2* values were calculated in a circular region of interest and were converted into liver iron concentration (LIC) values. The T2* value in all the 16 cardiac segments was calculated and global value was the mean [3]. A T2* > 20 ms was taken as "conservative" normal value. Biventricular function parameters were quantified by cine images.

The mean global pancreatic T2* value was 12.12 ± 8.27 ms (range: 1.4–33.7 ms).

One-hundred thirty-seven patients (93.2%) had pancreatic iron.

The pancreatic head had a significant higher T2* value than the body (12.28 ± 8.88 ms vs. 11.34 ± 7.79 ms; $P = 0.050$). The posterior-inferior part of the pancreatic head and the uncinate process derive from the ventral pancreatic anlage while the other pancreatic regions originate from the dorsal anlage. These two parenchymal portions differ in many histological and functional aspects and may load iron at a slight different extent. Clinical consequence of this different regional IO are of interest.

Global pancreatic T2* value was comparable between the sexes and it was not associated to age.

None of the teen patients without pancreatic IO had diabetes or impaired glucose tolerance (IGT). A normal global pancreas T2* value showed a negative predictive value of
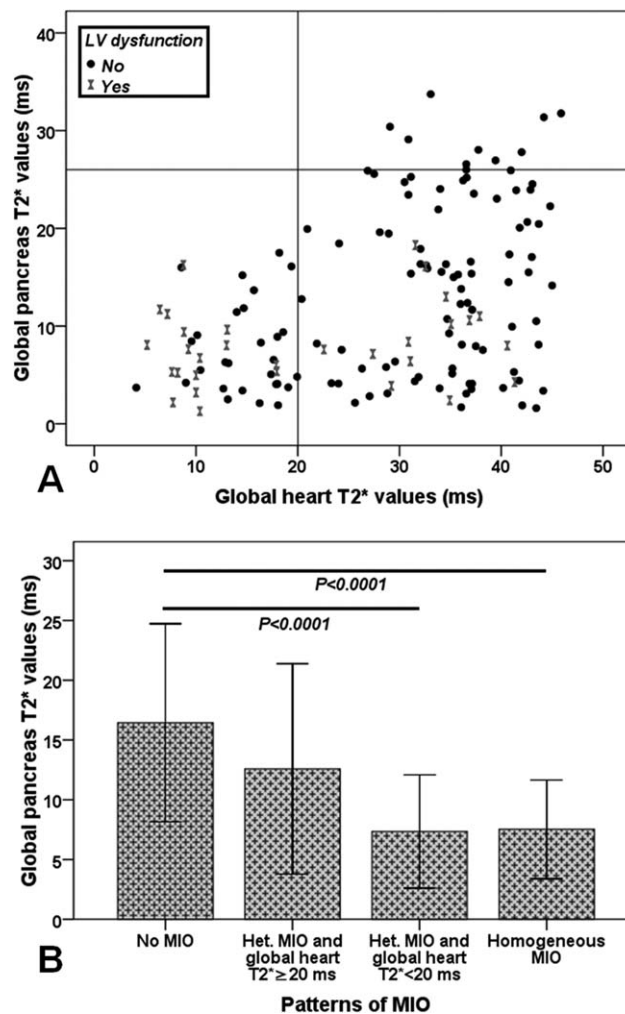

Figure 1. A: Scatter plot of global pancreas T2* values versus global heart T2*values. The horizontal and vertical dotted lines represent the cut-off for T2* values. **B**: Global pancreas T2* values in the 4 groups of patients with different patterns of MIO.

100% for disturbances of glucose metabolism. However, global pancreatic T2* values were comparable between patients without and with impaired glucose metabolism (12.54 ± 8.69 ms vs. 10.48 ± 6.18; $P = 0.503$). It has been shown that pancreatic atrophy (volume) was a better predictor of diabetic status than pancreatic hemosiderosis (T2*) and that endocrine failure was not statistically related to pancreatic T2* results [4]. Moreover, T2* measurements represent a punctual observation, providing information about the present. Conversely, pancreatic iron deposition is an early event and many patients may have initially normal glucose metabolism. Along the time the iron-mediated oxidative stress triggers apoptosis, volume loss, and fatty replacement, leading to pancreatic dysfunction [5,6]. Furthermore, to interpreter our findings, we must consider that although it is known hat iron accumulates selectively within beta-cells and that the progression of IGT toward overt diabetes mellitus depends on the severity and duration of severe IO, the pathogenetic mechanisms leading from siderosis to the development of diabetes are still poorly understood.

There was a significant negative correlation between serum ferritin levels and global pancreatic T2* values ($R = -0.341$, $P < 0.0001$). No patient with a serum ferritin level ≥ 2500 ng/ml had a normal global pancreas T2* value.

A significant inverse correlation was found between MRI LIC and pancreatic T2* values ($R = -0.329$, $P < 0.0001$). This datum differs from some previous studies [5]. Improved efficiency of T2* relaxometry compared with semiquantitative techniques over a wide range of IO and a larger population in our study may account for this discrepancy.

Global pancreas T2* values showed a significant positive correlation with global heart T2* values ($R = 0.330$, $P < 0.0001$; Fig. 1A) and the number of segments with normal T2* ($R = 0.346$, $P < 0.0001$). Of the 137 patients with pancreatic IO, 45 (32.8%) had a pathological global heart T2* value. No patients without pancreatic IO had MIO. A normal global pancreas T2* value showed a negative predictive value of 100% for cardiac iron.

The positive correlation between myocardial and pancreatic IO is more likely due to the same L-type calcium iron channels in the two organs, taking up circulating NTBI.

Four groups of patients were identified by the cardiac segmental approach: 42 patients (28.6%) showed no MIO (all segments with T2* > 20 ms), 60 patients (40.8%) showed an heterogeneous iron distribution (some segments with T2* > 20 ms and others with T2* < 20 ms) with global heart T2* ≥ 20 ms; 16 patients (10.9%) showed an heterogeneous MIO with global heart T2* < 20 ms, and 29 patients (19.7%) showed an homogeneous MIO (all segments with T2* < 20 ms). The global pancreas T2* was significantly higher in patients with no MIO than in patients with heterogeneous MIO and global heart T2* < 20 ms and in patients with homogeneous MIO (Fig. 1B). We showed for the first time ever that the association between cardiac and pancreatic haemosiderosis was true also considering a myocardial segmental analysis and the patterns of iron distribution.

There was a significant correlation between global pancreatic T2* values and LV EF ($R = 0.171$, $P = 0.039$). Out of the 31 patients with LV dysfunction, none showed a normal pancreatic T2* value (Fig. 1A). Global pancreas T2* values were significantly higher in patients with normal LV function than in patients with LV dysfunction (13.21 ± 8.75 ms vs. 8.05 ± 4.16 ms; $P = 0.013$). Conversely, pancreatic iron load was not significantly correlated with RV EF and this finding probably reflects the different RV sensitivity to the iron.

In conclusion, pancreatic IO was positively correlated to myocardial iron distribution and LV function. Pancreas T2* was a powerful predictor for heart iron burden and function.

We strongly recommend to incorporate in clinical practice T2* pancreatic measurements as prospective markers of cardiac iron risk. If a patient with no cardiac iron demonstrates pancreatic iron, it would be prudent to modify iron chelation therapy to prospectively prevent cardiac iron accumulation rather than wait for cardiac iron and dysfunction to appear.

Moreover, performing abdominal MRI could significantly reduce need of sedation in young patients, costs, and magnet time, particularly in countries where it is difficult to perform cardiac MRI.

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Fertility in transfusion-dependent thalassemia men: Effects of iron burden on the reproductive axis

To the Editor: Decreased reproductive capacity in transfusion dependent thalassemia (TDT) men is common but addressed infrequently. Previous reports estimate that more than one half of men with TDT are affected by oligospermia and asthenospermia and have abnormal sperm quality due to transfusion-induced iron overload [1,2]. Hypogonadotrphic hypogonadism and subfertility due to increased iron in the pituitary gland has been reported [3]. However, additional mechanisms affecting reproduction in TDT men and progression to infertility as it relates to systemic and pituitary iron load are not fully understood, delaying efforts for effective intervention. Oxidative stress, through increased production of reactive oxygen species (ROS), is considered a major contributory factor to male infertility causing damage to the sperm membrane, nucleus, and proteins, thereby impairing sperm quality [4]. Normal seminal plasma contains defense mechanisms against ROS consisting primarily of glutathione (GSH) as well as carnitine, folate, vitamins E, C, and A, zinc, and selenium [5]. In iron-overload thalassemia patients, excess non-transferrin bound iron (NTBI) and labile plasma iron (LPI) can act as pro-oxidants, resulting in increased generation of ROS. This coupled with low antioxidant defense molecules was suggested as a basis of tissue damage and impaired organ function in thalassemia [6,7]. Whether abnormalities involving these biochemical pathways are the basis for abnormal spermatogenesis and subfertility in thalassemia men with a high iron burden is not well studied. Additionally, the extent of iron-induced pituitary damage resulting in low gonadotropins and affecting reproduction capacity is not known. Thus, we investigated the pituitary-gonadal axis in a subset of TDT patients in the context of their total iron burden.

All TDT men, 18 years and older, at our thalassemia center were approached about the study, there was no preselection. Seven TDT men and two normal controls were studied. Anterior pituitary volume and iron accumulation (R2) were measured by pituitary MRI and corresponding Z scores calculated. Sperm count and motility were determined and sperm DNA integrity (DNA fragmentation Index; DFI) was assessed using Sperm Chromatin Structure Assay. Semen plasma elemental content was analyzed by inductively coupled plasma optimal emission spectrometry and GSH was quantified using a liquid chromatography linked tandem mass-spectrometry (LC/MS/MS) assay. NTBI/LPI were analyzed (London, England) and liver iron concentration (LIC), cardiac T2* MRI, ferritin, vitamins C and E, and plasma zinc levels reviewed.

All patients (median 26, range 21–30 years) went through spontaneous puberty at age 13–16. Retrospective analysis showed consistent low iron burden in two patients (1 and 4) and variable iron overload in the others (Table I). Mean pituitary iron (R2) was increased and mean anterior pituitary volume was decreased compare to normal age-matched male controls: R2 16.2 ± 2 vs. 11.2 Hz ($Z = 6.0$); 329.6 ± 83.3 vs. 596.1 ± 94.4 mm^3 ($Z = -3.0$), respectively. Anterior pituitary volume and LH level correlated with sperm count. Five of the seven patients (70%) had either low sperm count or complete azoospermia and variable sperm motility (Table II), an important measure of fertilization capacity. DFI was normal indicating no increase in sperm DNA fragmentation. Seminal plasma analysis revealed elevated iron concentration in 5/6 patients, highest in the three patients with azoospermia while iron was undetected in the normal controls. Seminal iron was inversely associated with sperm count and motility (Table II). The three patients with azoospermia, also exhibited low seminal plasma zinc levels despite a normal plasma range, as well as the lowest GSH concentration, inversely correlating with systemic iron overload. Plasma vitamins C and E were within the low-normal range (not shown) and zinc levels were in low or low-normal range (62–97; nl 70–120 mcg/dL).

Our results elucidate causes of infertility in NTDT men; we found high seminal iron in most patients, associated with azoospermia or oligospermia that to the best of our