Iron load

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Abstract

Recent research addressed the main role of hepcidin in the regulation of iron metabolism. However, while this mechanism could be relevant in causing iron load in Thalassemia Intermedia and Sickle-Cell Anemia, its role in Thalassemia Major (TM) is marginal. This is mainly due to the high impact of transfusional requirement into the severe increase of body iron. Moreover, the damage of iron load may be worsened by infections, as HCV hepatitis, or liver and endocrinological damage. One of the most relevant associations was found between splenectomy and increase of risk for mortality due, probably, to more severe iron load. These issues suggest as morbidity and mortality of this group of patients they do not depend only by our ability in controlling heart damage but even in preventing or treating particular infections and complications. This finding is supported by the impairment of survival curves in patients with complications different from heart damage. However, because, during recent years different direct and indirect methods to detect iron overload in patients affected by secondary hemochromatosis have been implemented, our ability to maintain under control iron load is significantly improved. Anyway, the future in iron load management remains to be able to have an iron load map of our body for targeting chelation and other medical treatment according to the single organ damage.

Introduction

Iron metabolism is stringently regulated in our body. This is due because of its possible increase may determine severe organ damage. The total iron body content is from 3 to 4 grams (1). Haemoglobin approximately contains 2.5 grams of iron (1). Iron containing proteins is 400 mg (1). Iron bound to transferrin in plasma is from 3 to 7 mg, while the remainder storage is contained as iron ferritin or hemo-

siderin proteins (1). Iron is lost in sweat, shed skin cells, and perhaps some gastrointestinal loss at a rate of approximately 1 mg/day (1). Recent research has been addressed as hepcidin has a central role in iron homeostasis (2). The main role of hepcidin, secreted into the circulation, is to downregulate the ferroportin-mediated release of iron from enterocytes, macrophages and hepatocytes (Figure 1) (2). Actually, iron is reduced to the ferrous state by duodenal ferric reductase (DcYtb), it is transported into the cells by divalent metal transporter 1 (DMT1) and released by way of ferroportin (Figure 1) (1). Moreover, hepatocytes take up iron from circulation either as free iron or transferrin-bound iron (1). Furthermore, transferrin receptor 2 may serve as a sensor of circulating transferrin-bound iron (Figure 1) (1).

This mechanism of iron overloading could be relevant in patients with thalassemia intermedia and sickle-cell anemia, but it is not crucial in patients with thalassemia major. In this cohort of patients iron body burden is determined by transfusional requirement. As we know, transfusional regimen, to maintain pre-transfusion haemoglobin level around 10 g/dl, was shown to be able to promote normal growth, to give a good quality of life, to avoid or delay splenectomy, and to minimize iron overload (3). Calculation of annual blood requirement and transfusional iron loading by regular recording of transfused blood for each patient is necessary for monitoring the hypersplenism and to establish a tailored chelation treatment, possible today thanks to the availability of different iron chelators. However, one unit of transfused blood contains around 200-250 mg of iron (4). Moreover, iron accumulates with repeated infusions. Therefore, chronic transfusion-dependent patients have an iron excess of ~0.4-0.5 mg/kg/day (1 g/month) and first signs of iron overloading can be seen after 10-20 transfusions (4). Finally, the severe increase of iron in our body, causing severe organ damage, is followed from high impact on morbidity and mortality.

Therefore, hypogonadism (about 50%), hypothyroidism (about 10%) and hypoparathyroidism (about 9%) remain some of the most common complications and hormone replacement therapy (HRT) is the only way to treat these. Moreover, insulin dependent diabetes affects several thalassemia patients, not considering that impairment of β -cell function (as determined by SC Homa) is detected earlier (5). Severe Growth Hormone Deficiency (GHD) was recently detected in 25% of adult patients with major or intermedia thalassemia, being in these patients the mean femoral T-score significantly lower ($p < 0.01$). Post-transfusional viral infections affected patients transfused before introduction of screening tests on donor blood and are mainly related to HIV (less than 2%) and HCV (up to 70%) (6). Moreover, transfusional liver iron overloading together with HCV hepatitis increases the risk of fibrosis evolution (6). Therefore, although the most efficacious antiviral therapy for chronic HCV hepatitis is today based on the association of Peg-Interferon plus Ribavirin, with cumulative Sustained Virological Response (SVR) over 45% (obtained in a small cohort of thalassemia patients), the extension of this antiviral combined treatment is actually delayed by the secondary hemolytic anemia due to ribavirin (6). Moreover, the patients with clinical and/or histological diagnosis of cirrhosis are about 8% (7) of risk for hepatocellular carcinoma (HCC) and, for this reason, they periodically have to receive appropriate screening (8).

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It is well known as cardiac disease-free survival is related to the frequency of serum ferritin levels less than 2500 ng/ml. This means that patients with more than 67% of ferritin levels >2500 ng/ml have poor prognosis, while patients with less than 33% of ferritin levels >2500 ng/ml have nice prognosis. Intermediate prognosis was found in patients with thalassemia major with 33 to 67% of ferritin levels determinations >2500 ng/ml (Figure 2) (9).

Recently, it was shown as even past-heart failure, cirrosish and arrhythmia together with other complications, including endocrinological complications are related with higher risk for mortality on separate Cox regression model (Figure 3) (10). One of the most relevant associations was that between splenectomy and increase of risk for mortality (Figure 3) (10). This finding suggests as this organ could be crucial to decrease the risk for mortality because its high capacity of iron storage without any risk for tissue damage. This result could induce to reevaluate needing for splenectomy, especially in countries where blood transfusion requirement, to maintain haemoglobin level >9.5 g/dl can be obtained. Moreover, Kaplan-Meier curves for single complication were obtained, suggesting as impairment of survival curves in thalassemia major does not depends only by cardiac disease (Figure 4).

These findings suggest as the main aim of treatment managing in thalassemia major patients should be to prevent organ damage as liver and endocrinological diseases together with high serum ferritin levels. Moreover, they suggest as the way to obtain this goal is not only to manage chelation treatment for maintaining, as long as possible, serum ferritin levels <2500 ng/ml but even to make all other interventions necessary to prevent organ damage (*i.e.* HBV vaccination, regular blood transfusions, endocrinological survey, etc.).

During recent years, different direct and indirect methods to detect iron overload in patients affected by secondary hemochromatosis have been implemented (Figure 5).

Among these, serum ferritin levels determination is so far the most worldwide available tool. However, although this procedure is easy and available, it does not correlate with Liver Iron Concentration (LIC) in single cases (Figure 5).

In recent years, non-invasive methods to measure liver iron overload have been studied. Noninvasive methods for measuring liver iron overload, such as biosusceptometry by superconducting quantum interference device systems and magnetic resonance imaging (MRI), have been evaluated in thalassemia and hemochromatosis patients. After the initial report in 1982 (11), subsequent studies of superconducting quantum interference device biosusceptometry in clinical applications were limited to only a few specialized centres (12).. A strong correlation was demonstrated between liver iron concentration by biopsy and a quantitative measurement of the MRI signal amplitude using the R2 or R2* methodology (Figure5) (13-15). These evidences on accuracy of non-invasive methods for assessment of liver iron concentration are sufficient to consider MRI-R2 methodology as a worldwide available alternative to liver biopsy for liver iron measurement. However, liver biopsy is still considered the *gold standard* for the evaluation of liver damage and is recommended for the assessment of HBV or HCV chronic hepatitis by international guidelines (Figure5) (16).

To date the gradient echo T2* technique is the most robust method, allowing sensitive, rapid and reproducible quantification of myocardial iron (17-19) (Figure 5). The most recent multiecho (single breath-hold) T2* *versus* the single echo (multi breath hold) T2* technique has permitted faster and more reproducible exams (18).

Thus, using a multislice multiecho T2* approach it has been possible to extend myocardial iron evaluation from the mid-ventricular septum to all left ventricle by a segmental approach (19-20). In fact, histological and MRI studies have previously demonstrated heterogeneous myocardial iron distribution. The multislice multiecho T2* approach accounting for the heterogeneous myocardial iron distribution has permitted to identify three groups of patients (homogeneous, heteroge-

neous, and no myocardial iron overload) that are statistically different in serum ferritin levels and liver iron concentration (19).

Recently, Kirk P, *et al.* (21) suggested as T2* values <10 ms (Relative Risk 159, P <0.001) and T2* <6 (RR 268, P <0.001) were associated with a significantly increased risk of heart failure in comparison with cardiac T2* values >20 ms (Figure 6).

Figure 1. Hepcidin control of iron homeostasis: minor role in thalassemia major.

Figure 2. Cardiac disease-free survival corresponding to different percentages of serum ferritin levels exceeding 2500 ng/ml (Olivieri *et al.*,1994).

COMPLICATION	HR(95% CI)	p-value	N. SUBJECTS (DEATHS)
PAST-HEART FAILURE	10,7(5,8;19,7)	<0,0001	357(41)
CIRROSHIS	8,1(3,9;16,6)	<0,0001	341(31)
ARRHYTHMIA	6,8(3,8;12,1)	<0,0001	367(47)
DIABETES	5,3(3,1;9)	<0,0001	383(59)
HYPOPARATHYROIDISM	5,1(2,3;11,4)	<0,0001	332(28)
CARDIOPATHY	3,9(2,1;7,4)	<0,0001	174(41)
HYPOTHYROIDISM	3,4(1,6;7,3)	0,001	346(28)
HYPOGONADISM	2,9(1,3;6,5)	0,01	348(31)
SPLENECTOMY	2,8(1,6;4,9)	<0,0001	379(53)
MEAN FERRITIN (<2500 VS ≥ 2500)	4,2(2,6;6,5)	<0,0001	417(77)

Figure 3. Summary of overall survival based on separate cox regression models for single complication (Maggio *et al.*, 2012,unpublished data).

Moreover, cardiac echo-doppler has a prominent value either in evaluating the severity of right and left heart dysfunction and in identifying prognostic factors of heart failure. Recently, our group suggested as reduction in LVEF $\geq 7\%$, over time, determined by 2-D echocardiography, may be considered a strong predictive tool for the detection of thalassemia major patients with increased risk of cardiac death (Figure 7, Table 1) (submitted paper). The reduction of LVEF% $\geq 7\%$ had higher (84.76%) predictive value. Finally, Kaplan-Meier survival curves of thalassemia major patients with LVEF $\geq 7\%$ showed a statistically significant decreased probability of survival for heart disease ($p = 0.0022$) (Figure 8).

Finally, different approaches have been performed to detect iron overloading in different organs as pituitary gland, brain, pancreas, adrenals, spleen, lymph node and bone marrow. Moreover, the possibility of detecting single organ iron body burden is the main challenge for the future and it could change the clinical approach to chelation treatment in thalassemia major patients, targeting specific chelation for single organ damage. However, none of the study, reported on literature and concerning the iron overloading evaluation of these organs, suggests, so far, that MRI of organs different from liver and heart could be performed with sufficient reliability and reproducibility on routinely standard clinical practice.

Figure 4. Kaplan-Meier survival curves in thalassemia major patients for single complication (Maggio *et al.*, 2012, unpublished data).

	METHODS	EVALUATION
	FERRITIN	<ul style="list-style-type: none"> Easily, available Useful as an index of compliance with therapy Does not correlate with LIC in single cases
INDIRECT	TRANSFERRIN SATURATION	<ul style="list-style-type: none"> Useful in genetic hemochromatosis and thalassemia intermedia Not useful in thalassemia major
	LIVER BIOPSY	<ul style="list-style-type: none"> "Gold standard" for the evaluation of liver damage (International Guidelines) It is invasive, accuracy for LIC determination in presence of fibrosis and cirrhosis could be lower
	NMR (MRI):R2* and T2*	<ul style="list-style-type: none"> R2* is the "gold standard" procedure for LIC T2* is the only available tool to evaluate heart iron
	SQUID	<ul style="list-style-type: none"> Reproducibility in very specialized centers Available only in few centers

Figure 5. Direct and indirect methods to detect iron overload in patients affected by secondary hemochromatosis.

Figure 6. Lower heart T2* values are associated with increased risk for heart failure (Kirk *et al.*, 2009).

Figure 7. Estimated profiles from the fitted GEE model for the two patient-groups: the not alive (red color) versus the alive-group (black color) (Maggio *et al.* 2012, submitted).

Figure 8. Kaplan-Meier curves for cardiac disease mortality in the three categorized Thalassemia Major groups. The 2 category was represented from patients with a LVEF >7% during the time.

Table 1. GEE model to evaluate changes in mean ejection fraction levels in not alive-group versus alive-group over time.

	Coefficients (SE [*])	95% CI [*]	p-value [*]
Intercept	60,89 (0,61)	(59,69;62,09)	<0,0001
Status ^{**}	-6,58 (1,78)	(-10,08;-3,07)	<0,0001
Year ^{**}	0,27 (0,13)	(-0,001;0,54)	0,051
Status×Year ^{**}	-1,51 (0,41)	(-2,31;-0,71)	<0,0001

SE^{*} = Standard Error, CI^{*} = Confidence Interval, P-value^{*} from null hypothesis coefficient = 0 by Wald's test. Status^{**} is the variable indicating if a patient is dead or alive; Year^{**} is the variable indicating the consecutive years of observation, corresponding to t= 0, 1, 2, 3, 4; Status×Year^{**} is a status-by-time interaction effect.

Conclusions

In conclusion, although iron homeostasis is mainly controlled by hepcidin secretion, its role in the iron loading of thalassemia major patients is not relevant and transfusion remain the main cause of organ damage in this cohort of patients.

Therefore, the control of this has a central role in the management of thalassemia major patients. The aim of our treatment should be addressed to prevent all possible organs damage, considering that some of them have highest impact on the impairment of survival curves of these patients.

Moreover, the general clinical management of all other possible factors that may impair organ functionality should accompany the control of iron body burden. This approach is today possible thanks to the improvement of sensibility and specificity of non-invasive methods to measure liver and heart iron overloading.

The future in iron load management remains to be able to have an iron load map of our body for targeting chelation and other medical treatment according to the single organ damage.

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