Optimizing the management of hereditary haemochromatosis: the value of MRI R2* quantification to predict and monitor body iron stores

Hereditary haemochromatosis (HH) is a major cause of iron overload. Without therapeutic intervention, HH patients are at risk of severe tissue damage due to iron toxicity. HH-related morbidity and mortality can be effectively reduced by regular venesection (Niederau *et al*, 1985; Crosby, 1991), in the form of a course of weekly intensive phlebotomies (IPh) to deplete iron stores, followed by maintenance phlebotomies (MPh) to prevent iron re-accumulation (European Association for the Study of the Liver, 2010).

In spite of being a well-accepted, efficient and safe procedure, phlebotomies may raise concerns regarding limitations of patients' daytime activities, decreasing their quality of life (Brissot *et al*, 2010). To manage patient expectation at diagnosis and optimize treatment compliance, it is important to predict the amount of mobilised iron and expected IPh duration, and to define reliable and safe endpoints for serum ferritin (SF) levels during the MPh. In this setting, low SF values (50–100 µg/l are usually advocated in clinical practice (European Association for the Study of the Liver, 2010), although normal SF (males: <300 µg/l; females: <200 µg/l) have also been proposed (Vanclooster *et al*, 2016). Whether iron re-accumulation differs according to different SF endpoints has not been reported to date.

We have recently implemented a 3 Tesla multi-echo chemical shift-encoded gradient-echo magnetic resonance (MECSE-MR) sequence, with high accuracy to simultaneously quantify liver steatosis, assessed as proton density fat fraction (PDFF), and iron overload, assessed by R2* measurements (Martí-Bonmatí *et al*, 2011; França *et al*, 2017a,b). The same images also allow evaluation of the distribution of iron deposits in different abdominal tissues (França *et al*, 2017b). This method might be useful for predicting mobilizable total body iron stores (TBIS) during IPh and for assessing liver iron re-accumulation in MPh.

We recruited 48 phenotypically and genetically well-characterized HH patients (39 males, 15 females; age 19–80 years; 12 receiving IPh and 38 under MPh, 2 of which were also tested during IPh) and 21 apparently healthy controls (12 males, 9 females; age 20–77 years).

TBIS were quantified in all 12 HH patients undergoing IPh, as previously described (Porto *et al*, 1997), considering the total amount of iron (in grams) removed by phlebotomies from the date of MR examination until iron depletion, defined as SF <50 µg/l. As patients were recruited at different IPh stages (3 of them had a second MR examination after total depletion), the calculated TBIS values ranged from 0 to 12·3 g. Notably, there was no significant correlation between initial SF values (retrieved from patients' data at diagnosis) and the TBIS quantified by phlebotomy ($P = 0.578$), possibly due to the generally poor specificity of high SF values, and the fact that, at diagnosis, values were distributed within a limited range of severe iron overload. Nevertheless, SF values determined at several time points during iron depletion (i.e. with a wide range of values from iron overload to iron depletion) were significantly correlated with the calculated TBIS at the same time points ($R_S = 0.916$, $P < 0.01$). Therefore, SF appeared to be a good marker for monitoring iron depletion during IPh but could not predict TBIS at diagnosis.

Using the data from the patients undergoing IPh, a stepwise regression analysis was performed considering TBIS as dependent variable, taking gender, age and all R2* tissue determinations (liver, pancreas, spleen and vertebra bone marrow) as independent variables (Table I). Forward and backward selection methods were applied to select the most parsimonious model for predicting TBIS. The best regression model included two predictors: liver R2* (F-to-enter = 72·5553, $P < 0.0001$) and pancreas R2* (F-to-enter = 19·5504, $P = 0.0008$), with a coefficient of determination of $R^2 = 94.2\%$ ($P < 0.01$), being expressed by the equation: TBIS = 0·006 × Liver R2* + 0·009 × Pancreas R2* − 1·064. This new TBIS variable was designated FELIPA, for iron (FErrum) in liver (LI) and pancreas (PA).

Tissue iron quantification and distribution using R2* metrics was next assessed in the group of 38 patients undergoing MPh every 3 months for at least 2 years, with no change in intervention provided that SF values were maintained below the normal upper limit (males: 300 µg/l; females: 200 µg/l). In this group, SF values ranged from 9 to 300 µg/l, and were, in general, significantly correlated with hepatic iron deposits as assessed by liver R2* ($R_S = 0.611$, $P < 0.05$). No significant correlation was found between time since the end of IPh (range: 2–28 years) and SF or liver R2* values.

The "accepted standard" SF threshold of 99 µg/l (European Association for the Study of the Liver, 2010) identified patients with hepatic R2* higher than 48 Hz (the average R2* value in controls), with 87·5% sensitivity and 85·7%

Table I. Regression results. Regression results showing different test models to estimate total body iron stores (TBIS; dependent variable) using the different tissue R2* measurements (liver, pancreas, spleen and bone marrow), age and gender as independent variables. The best-fitted model (with the highest adjusted-R^2, "Model 2") has two significant predictors (liver R2* and pancreas R2*), being expressed as TBIS = 0·006 × Liver R2* + 0·009 × Pancreas R2* − 1·064.

|	Regression model		
Independent variable	Model 1	Model 2	Model 3
Liver R2*	0·006* (0·003, 0·009)	0·006** (0·005, 0·007)	0·006** (0·005, 0·007)
Pancreas R2*	0·008* (0·004, 0·013)	0·009** (0·006, 0·013)	0·009** (0·005, 0·012)
Spleen R2*	−0·012 (−0·043, 0·019)		
Bone marrow R2*	0·010 (−0·010, 0·030)		
Age	−0·009 (−0·051, 0·032)		−0·010 (−0·046, 0·027)
Gender	−0·807 (−2·091, 0·477)		−0·807 (−1·867, 0·547)
Constant	−0·472 (−4·066, 3·122)	−1·064* (−1·652, −0·476)	1·121 (−1·680, −1·922)
R^2	0·956	0·942	0·949
Adjusted R^2	0·923	0·932	0·929

*$P < 0.05$; **$P < 0.01$
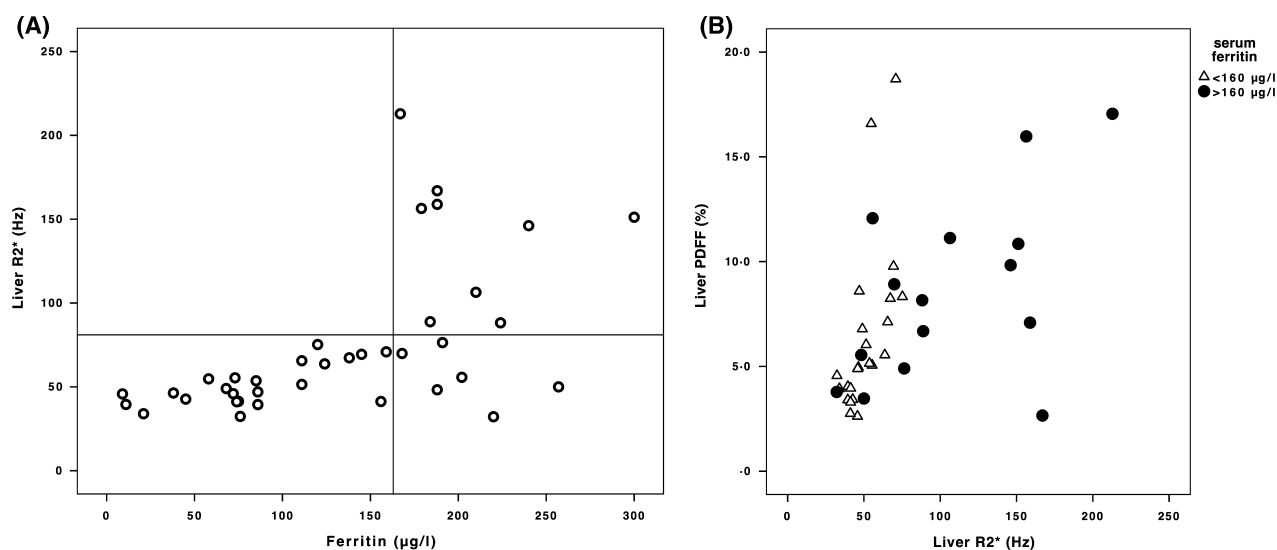
Fig 1. (A) Scatter plot of serum ferritin (SF) values and liver R2* values in patients with hereditary haemochromatosis (HH) under maintenance phlebotomy (MPh). Vertical lines indicate the SF cut-off value of 160 μg/l; the horizontal line indicates the highest hepatic R2* value in healthy controls, as reference. All patients with SF <160 μg/l had hepatic iron in the same range as healthy controls (100% sensitivity, 79·3% specificity). (B) Scatter plot of liver steatosis (proton density fat fraction [PDFF] values) and iron deposits (R2* values) in HH patients under MPh, grouped by SF values below or above the cut-off 160 μg/l. Patients with SF >160 μg/l had the highest hepatic R2* and PDFF values, suggesting higher accumulation of iron and steatosis, respectively.

specificity. A SF threshold of 160 μg/l, in turn, identified patients with hepatic R2* higher than 81 Hz (the highest R2* value in controls) with 100% sensitivity and 79·3% specificity, thus supporting this threshold as a safe value to assume hepatic R2* within normal limits (Fig 1A). Importantly, liver steatosis, defined as PDFF >4·8% (França et al, 2017a), was a common finding (67% of MPh patients), particularly when SF was >160 μg/l(80% of these patients) (Fig 1B). This association of liver steatosis with high SF values can be interpreted as steatosis itself giving rise to an increased SF independently of iron load, but it does not rule out the hypothesis that minimal iron toxicity might also be involved in the pathogenesis of steatosis.

Our study had limitations. The small number of IPh patients to quantify TBIS hampers the construction of predictive models extensible to larger populations. Although methodological constraints limit iron quantification above a certain load, the iron range observed in this study corresponds to the range of values where more precision is needed in daily clinical practice. A SF threshold for normal liver R2* can be very useful as a practical cut-off, but it does not determine whether iron-induced tissue damage could still be occurring (e.g., as a result of abnormally increased non-transferrin-bound iron).

In conclusion, MR determined R2* measurements as surrogate markers of TBIS in patients with HH are feasible.

FELIPA, a new variable obtained from liver and pancreas R2* measurements, was correlated with TBIS, calculated by quantitative phlebotomies. FELIPA might be a new useful tool for estimating the severity of iron burden and managing patients' expectations on IPh duration. Liver R2* measurements in HH patients undergoing MPh suggest a best SF cut-off value of 160 µg/l to discriminate between patients with normal iron stores and those in whom there is iron re-accumulation.

Conflict of interest

Luis Martí-Bonmatí and Ángel Alberich-Bayarri are co-founders of QUIBIM SME. The remaining authors declare that they have no conflicts of interest.

Authors contribution

Manuela França, Graça Porto, Angel Alberich-Bayarri and Luis Martí-Bonmatí designed the research; Manuela França, Sara Silva, Filipa Vilas Boas, Helena Pessegueiro and Graça Porto performed data acquisition; Manuela França, Graça Porto, Carla Oliveira and Luis Martí-Bonmatí analysed and interpreted the data; Manuela França, Luis Martí-Bonmatí and Graça Porto wrote the paper. Sara Silva, Carla Oliveira, Filipa Vilas Boas, Angel Alberich Bayarri and Helena Pessegueiro Miranda reviewed the paper critically; All the authors approved the submitted version.

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