Mediterranean Journal of Hematology and Infectious Diseases

Original Article

Liver Iron Content in Individuals With B-Thalassemia Trait and Hyperferritinemia: Role of Metabolic Alterations, *HFE* Genotypes, and Cirrhosis

Giulia Risca[1,2], Raffaella Mariani[3,4], Mara Botti[3,4], Sara Pelucchi[2], Stefania Galimberti[1,5] and Alberto Piperno[4,6].

[1] Bicocca Bioinformatics Biostatistics and Bioimaging B4 Centre, University of Milano-Bicocca, Monza, Italy.
[2] Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy.
[3] Rare Disease Unit, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy.
[4] European Reference Network for Rare Haematological Diseases, EuroBloodNet.
[5] Biostatistics and Clinical Epidemiology, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy.
[6] Centro Ricerca Tettamanti, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italia.

Competing interests: The authors declare no competing interest.

Abstract. ***Background:*** **An increased serum ferritin is a frequent finding in adults with β-thalassemia trait (BTT). However, whether such an increase is associated with a proportional increase in iron stores is unclear.**
Objectives. **We aimed to evaluate liver iron stores in a consecutive cohort of BTT with hyperferritinemia who underwent magnetic resonance imaging (LIC^{MRI}) for clinical purposes.**
Methods. **Sixty-six BTT subjects with hyperferritinemia were studied. Clinical, biochemical, and genetic evaluations were done to assess the cause of hyperferritinemia. LICMRI was classified as: grade-1= ≤3 mg/g (normal/mild); grade-2= >3≤7 mg/g (moderate); grade-3= >7 (severe).**
Results. **80.3% showed normal/mild (n=29, 43.9%) or moderate LIC^{MRI} (n=24, 36.4%), while 19.7% (n=13) showed values >7 mg/g. The latter had lower haemoglobin concentration (p=0.004) and higher transferrin saturation and ferritin compared to subjects with lower LIC^{MRI} (p<0.001), while steatotic liver disease was more frequent in subjects with lower LIC^{MRI} grades (p=0.012). Liver cirrhosis was significantly more frequent in subjects with moderate/severe than in those with lower LIC^{MRI} grades (p=0.001 and p=0.025, respectively). We found a higher frequency of HFE and non-HFE iron-related genotypes (risk genotypes) in LIC^{MRI} grades 2-3 compared to none in LIC^{MRI} grade 1 (p=0.003 and p<0.0001, respectively). A regression analysis identified risk genotypes, liver cirrhosis, and BMI as significantly associated with LIC^{MRI}.**
Conclusions. **Hyperferritinemia is common in BTT subjects, but major iron overload is limited to a minority of cases. They present associated genetic and acquired causes of iron accumulation and increased risk of liver damage.**

Keywords: Beta-Thalassemia; Hyperferritinemia; Iron Overload; Liver; Cirrhosis.

Citation: Risca G., Mariani R., Botti M., Pelucchi S., Galimberti S., Piperno A. Liver iron content in individuals with β-thalassemia trait and hyperferritinemia: role of metabolic alterations, *HFE* genotypes, and cirrhosis. Mediterr J Hematol Infect Dis 2026, 18(1): e2026051, http://dx.doi.org/10.4084/MJHID.2026.051

Published: July 01, 2026 **Received: March 02, 2026** **Accepted: June 16, 2026**

Correspondence to: Prof. Alberto Piperno. Centro Ricerca Tettamanti - Fondazione IRCCS San Gerardo dei Tintori, Monza,

Introduction. About 1.5% of the world's population is a carrier of β-thalassemia (BTT). While the highest prevalence is in the traditional malaria-endemic regions, BTT is now encountered worldwide due to population migrations from high-prevalence areas.[1] Subjects with BTT show mild ineffective erythropoiesis, erythroid hyperplasia, and hepcidin suppression.[2–4] Some authors suggest that even slight hepcidin suppression may increase iron absorption, possibly leading to iron overload.[4-5] Indeed, serum ferritin levels were significantly higher in adults with BTT than in controls.[6–8] However, it is unclear whether this increase is proportional to liver iron stores, as none of these studies measured liver iron concentration (LIC). Recently, Busti et al.[9] studied 30 subjects with BTT and liver iron overload as assessed by MRI or liver biopsy. They found variable serum ferritin levels (from 441 to 3650 µg/L), transferrin saturation (TSAT) (from 28 to 100%), and amount of iron overload, from mild to severe. Other studies evaluated the role of *HFE* variants (p.C282Y and p.H63D) on serum ferritin levels in BTT, yielding conflicting results, mainly due to the small sample sizes and the lack of liver iron measurement.[10–14] By contrast, it was shown that the presence of BTT may contribute to the severity of iron overload in patients with *HFE*-hemochromatosis homozygous for the p.C282Y mutation[5] and the same was suggested for those carrying the p.H63D homozygous genotype.[10] However, although hyperferritinemia is quite common in BTT patients, there are still unclear questions: *i.* how many patients with BTT and hyperferritinemia have liver iron overload amounts at risk for liver damage *ii.* What role do concomitant factors (e.g., alcohol, metabolic alterations, HFE variants) play in favouring hyperferritinemia and/or iron overload in BTT. To achieve these objectives, we studied 66 consecutive subjects with BTT and hyperferritinemia who underwent liver iron quantification by magnetic resonance imaging (LIC^{MRI}) for clinical purposes.

Materials and Methods. From a large series of 684 subjects with hyperferritinemia (serum ferritin > 350 µg/L in men and > 200 µg/L in women) who underwent LIC^{MRI} quantification from January 2008 to October 2024,[15] we selected the 66 patients with BTT. All MRIs were performed at diagnosis, prior to iron depletion therapy, together with other investigations usually needed for the characterisation of hyperferritinemia.[16,17] Accurate clinical and biochemical evaluations were performed before the MRI. Age at time of MRI, sex, alcohol intake, body mass index (BMI), presence of arterial hypertension and diabetes, blood count, iron parameters (TSAT and ferritin), liver and metabolic indices (triglycerides, HDL cholesterol, glycemia) were collected. Subjects with a chronic history of alcohol consumption ≥30 g/day in men and ≥20 g/day in women were classified as alcohol drinkers. BMI cut-off points for overweight and obesity were >26 kg/m^2 in men and ≥25 kg/m^2 in women, and ≥30 kg/m^2, respectively, and those for metabolic indices were as reported elsewhere.[18] The presence of liver steatosis was assessed by abdominal ultrasound by an expert internal medicine sonographer. Subjects were further classified according to the EASL-EASD-EASO guideline for Metabolically active steatotic liver disease (MASLD).[18] In subjects with steatotic liver disease (SLD), chronic hepatitis, high alcohol intake, and marked iron overload, the assessment of liver damage (e.g., severe fibrosis/cirrhosis) was done by liver function tests, blood-based score (FIB-4),[18] abdominal ultrasound, at diagnosis and during follow-up, and fibroelastography and liver biopsy when needed. LIC^{MRI} was assessed as previously reported[19] and was graded according to the severity of iron overload: ≤3 mg/g (grade 1), >3 ≤7 mg/g (grade 2), and >7 mg/g (grade 3). Grade 1 included patients with normal or slightly increased LIC not deserving therapeutic intervention, grade 2 those with mild-moderate iron overload in which tailored iron depletion therapy could be considered but is not mandatory, and grade 3 those with moderate-severe iron overload worthy of therapeutic intervention.[20,21] Genotyping of iron-related genes was performed according to the current hemochromatosis guidelines.[22] According to the recent hemochromatosis classification recommendation,[23] we considered p.Cys282Tyr homozygosity, compound heterozygosity for p.Cys282Tyr and another pathogenic HFE variant, and digenic genotypes as genotypes able to favour iron overload (henceforth defined as risk genotypes). In addition, we also included in the group of risk genotypes, the p.His63Asp homozygous and p.His63Asp/p.Cys282Tyr genotypes based on the evidence that they can lead to hepatic iron loading in the animal model,[24] and can act as modifiers of iron phenotype in humans when coexisting with other conditions favoring iron overload.[5,9-10,23] The manuscript is in accordance with ethical standards stated in the 1964 Declaration of Helsinki and its later amendments. Informed written consent for molecular testing and data recording in the local database was obtained from all participants, and the collection was conducted in accordance with Institutional rules.

Statistical methods. Due to the skewed nature of continuous variables, median and 1st-3rd quartiles (Q1-Q3) were calculated for descriptive purposes, while qualitative variables were reported as absolute and relative frequencies. The Kruskal-Wallis rank-sum test and the Fisher's exact test were performed to compare LIC^{MRI} grades, as appropriate, and were adjusted for multiple comparisons using the Benjamini-Hochberg

Table 1. Data of subjects with β-thalassemia trait according to LIC^{MRI} grades.

Characteristic	Level	Overall n=66	LIC^{MRI} Grade 1 n=29 (43.9%)	Grade 2 n=24 (36.4%)	Grade 3 n=13 (19.7%)	p-value
Sex	F	7 (10.6)	4 (13.8)	2 (8.3)	1 (7.7)	0.877
	M	59 (89.4)	25 (86.2)	22 (91.7)	12 (92.3)	
Age at MRI (years)		53.5 (43.0-58.8)	51.0 (39.0-58.0)	53.5 (46.5-57.0)	57.0 (47.0-67.0)	0.373
Heavy drinker	No	50 (75.8)	24 (82.8)	18 (75.0)	8 (61.5)	0.340
	Yes	16 (24.2)	5 (17.2)	6 (25.0)	5 (38.5)	
Hypertension	No	44 (66.7)	18 (62.1)	15 (62.5)	11 (84.6)	0.360
	Yes	22 (33.3)	11 (37.9)	9 (37.5)	2 (15.4)	
BMI (kg/m^2)		25.7 (24.1-27.5)	26.8 (25.4-28.0)	25.9 (23.8-27.1)	24.3 (22.7-25.5)	0.002
Overweight/ Obesity	No	32 (48.5)	9 (31.0)	12 (50.0)	11 (84.6)	0.005
	Yes	34 (51.5)	20 (69.0)	12 (50.0)	2 (15.4)	
MCV (fL)		64.7 (62.6-67.6)	63.5 (61.60-66.30)	66.4 (63.97-69.05)	64.9 (63.3-67.1)	0.054
Platelets (103/μL)		201.0 (163.0-241.8)	183.0 (163.0-235.0)	223.0 (175.3-270.3)	206.0 (161.0-256.0)	0.329
Transferrin saturation (%)		48.3 (35.4-63.3)	40.1 (26.2-48.5)	50.6 (43.8-58.8)	81.7 (74.0-90.3)	<0.001
Ferritin (μg/L)		939.5 (645.8-1341.3)	873.0 (679.0-1048.0)	829.5 (609.8-1345.5)	1459.0 (1261.0-2127.0)	<0.001
ALT* (U/L)		32.0 (25.0-50.3)	35.5 (25.8- 56.0)	29.0 (24.0- 38.0)	35.0 (24.0-56.0)	0.414
γ-GT* (U/L)		29.0 (21.0-56.0)	28.0 (23.5- 74.8)	29.0 (20.0- 43.0)	30.5 (24.5-45.0)	0.610
HCV	No	64 (97.0)	28 (96.6)	24 (100.0)	12 (92.3)	0.486
	Yes	2 (3.0)	1 (3.4)	0 (0.0)	1 (7.7)	
Glycemia* (mg/dL)		93.0 (84.0-105.5)	92.0 (83.8-99.0)	91.0 (86.0-106.8)	98.0 (90.0-110.0)	0.438
HDL Cholesterol* (mg/dL)		41.0 (35.3-51.8)	40.5 (37.0-49.5)	41.5 (33.5-50.8)	42.0 (32.5-52.3)	0.572
Triglycerides* (mg/dL)		110.5 (82.8-142.8)	108.0 (74.0-192.0)	126.0 (95.0-138.5)	101.5 (83.8-114.3)	0.261
SLD (%)	No	29 (43.9)	7 (24.1)	13 (54.2)	9 (69.2)	0.012
	Yes	37 (56.1)	22 (75.9)	11 (45.8)	4 (30.8)	
Liver cirrhosis	No	55 (83.3)	28 (96.6)	21 (87.5)	6 (46.2)	<0.001
	Yes	11 (16.7)	1 (3.4)	3 (12.5)	7 (53.8)	
Hyperglycemia	No	47 (71.2)	23 (79.3)	16 (66.7)	8 (61.5)	0.433
	Yes	19 (28.8)	6 (20.7)	8 (33.3)	5 (38.5)	
Hypertriglyceridemia	No	50 (75.8)	19 (65.5)	19 (79.2)	12 (92.3)	0.163
	Yes	16 (24.2)	10 (34.5)	5 (20.8)	1 (7.7)	
Hypocholesterolemia HDL	No	37 (56.1)	15 (51.7)	15 (62.5)	7 (53.8)	0.727
	Yes	29 (43.9)	14 (48.3)	9 (37.5)	6 (46.2)	
Metabolic alterations	0	13 (19.7)	3 (10.3)	6 (25.0)	4 (30.8)	0.192
	1	17 (25.8)	6 (20.7)	6 (25.0)	5 (38.5)	
	≥2	36 (54.6)	20 (69.0)	12 (50.0)	4 (30.8)	
Risk genotypes	No	51 (77.3)	29 (100)	17 (70.8)	5 (38.5)	<0.001
	Yes	15 (22.7)	0 (0)	7 (29.2)	8 (61.5)	

Legend: Results are reported as median (1st and 3rd quartile) or number (%), as appropriate. SLD= steatotic liver disease; * ALT, γ-GT, Glycemia, HDL Cholesterol, Triglycerides had 3.0%, 13.6%, 4.5%, 6.1% and 3% of missing values, respectively.

method. A linear regression model was performed to evaluate the relationship between LIC^{MRI} and risk genotypes, alcohol intake, BMI, SLD, and liver cirrhosis. The effect of each factor was first evaluated alone in univariate models and then combined in a multivariable model to consider their additive effect. All tests were two-sided with a significance level of 0.05. All analyses were performed using R (Version 4.4.3, www.r-project.org).

Results. Table 1 shows clinical and biochemical data for subjects according to LIC^{MRI} grade. The majority showed

normal/mild (n=29, 43.9%) or moderate LIC^{MRI} (n=24, 36.4%), and 13 (19.7%) showed LIC^{MRI} above 7 mg/g. There was a marked prevalence of men in all the classes. There were two patients with HCV-related chronic hepatitis, one with associated metabolic syndrome, and MASLD showing mild iron accumulation (grade 1), and the second (grade 3) was a heavy alcohol drinker. They both had cirrhosis. A single patient had a history of about twenty transfusions during hospitalisation for multiple trauma. Subjects with higher LIC^{MRI} were slightly but not significantly older (p=0.373), had lower haemoglobin concentration (p=0.004), and higher TSAT and ferritin compared to subjects with lower LIC^{MRI} (p<0.001). **Figure 1** shows the distribution of TSAT and serum ferritin according to LIC^{MRI}: 84.6% of BTT subjects with grade 3 LIC^{MRI} had TSAT >60%, compared to 25% and 6.9% of those with grade 2 and 1, respectively; 84.6% of patients with grade 3 LIC^{MRI} had ferritin >1000 µg/L compared to 45.8% and 31.0% in those with grade 2 and 1, respectively. BMI was significantly higher in subjects with lower LIC^{MRI} grades than in those with grade 3 (p=0.002). In detail, 20/29 (69.0%) subjects with LIC^{MRI} grade 1 were overweight (14/29, 48.3%) or obese (6/29, 20.7%) compared to 12/24 (50%) and 2/24 (8.3%) of those with grade 2, and 2/13 (15.4%) and 0/13 of those with LIC^{MRI} grade 3, respectively (p=0.005). Accordingly, SLD was more frequent in the lower LIC^{MRI} classes(p=0.012). **Supplementary Table 1** reports the different categories of SLD according to LIC^{MRI}, showing a higher frequency of MASLD/MetALD in the lower LIC^{MRI} classes

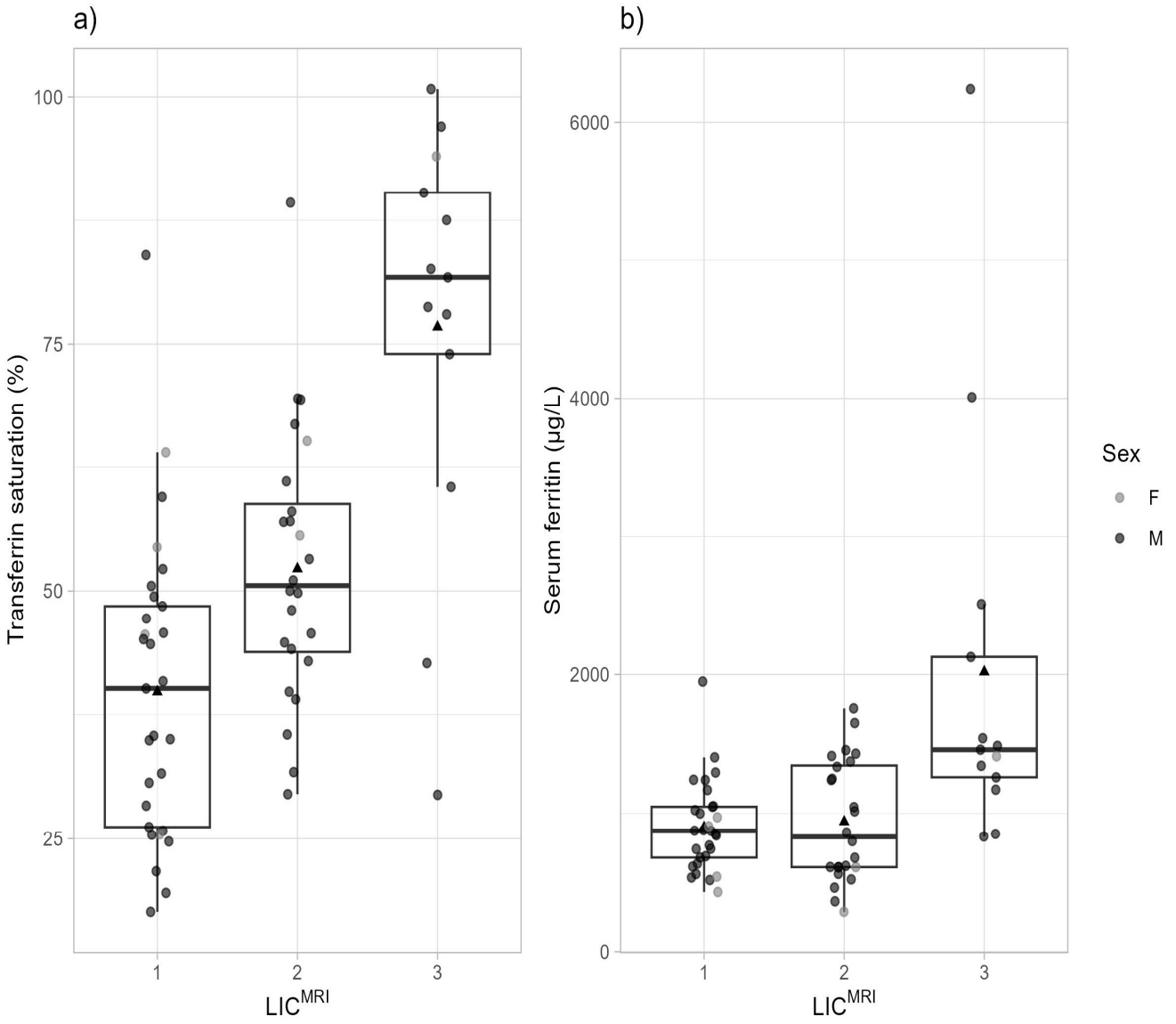

Figure 1. Transferrin saturation and serum ferritin. Box and whiskers plot of a) transferrin saturation (%) and b) serum ferritin (µg/L) according to LIC^{MRI} grades (●=Females, ●=Males). The median is represented by a horizontal line and means are indicated by black triangles (▲). LIC^{MRI}: Magnetic Resonance Imaging for Liver Iron Concentration.

Table 2. HFE and non-HFE in subjects with β-thalassemia trait according to LIC^{MRI} grades.

		LIC^{MRI}	
Genotype	**Grade 1 n=29**	**Grade 2 n=24**	**Grade 3 n=13**
HFE wildtype	19 (65.5)	11 (45.8)	3 (23.1)
HFE p.H63D +/-	7 (24.1)	4 (16.7)	2 (15.4)
HFE p.C282Y +/-	2 (6.9)	2 (8.3)	-
TFR2 p.M172K +/-	1 (3.5)	-	-
HFE p.H63D +/+	-	5 (20.8)	4 (30.8)
HFE p.C282Y +/+	-	1 (4.2)	3 (23.1)
HFE p.C282Y/p.Q233*	-	1 (4.2)	-
HFE p.C282Y +/- and TFR2 p.R712Q +/-	-	-	1 (7.7)

Legend: The different shades of gray show the different penetrance of genotypes.

Table 3. Clinical and genetic details of the 13 subjects with β-thalassemia trait with LIC^{MRI} >7 mg/g.

ID	Sex	Sex	LIC^{MRI} (mg/g)	Heavy drinker	BMI	Blood transfusion	Risk genotypes	Chronic hepatitis	Liver cirrhosis
1	1	M	7.2	yes	26.1	no	no	no	yes
2	2	M	7.6	yes	25.5	no	p.H63D/p.H63D	no	yes
3	3	M	10.3	yes	22.7	no	no	no	no
4	4	M	10.7	yes	24.6	no	p.H63D/p.H63D	no	yes
5	5	M	11.2	no	23.0	no	no	yes	yes
6	6	M	13.5	yes	21.9	no	no	no	no
7	7	M	13.5	no	16.8	no	p.H63D/p.H63D	no	no
8	8	M	14.2	no	23.8	no	HFE p.C282Y +/- and TFR2 p.R712Q +/-	no	yes
9	9	M	17	no	27.2	yes	no	no	no
10	10	M	21	no	18.4	no	p.H63D/p.H63D	no	no
11	11	F	23.4	no	24.3	no	p.C282Y/p.C282Y	no	no
12	12	M	27.6	no	24.7	no	p.C282Y/p.C282Y	no	yes
13	13	M	31.4	no	25.6	no	p.C282Y/p.C282Y	no	yes

Legend: M=Males; F=Females. The gray colored cells highlight the acquired and genetic factors favouring iron overload.

Table 4. Results of the univariate and multivariable linear regression models on LIC^{MRI}, respectively.

	Univariate model			Multivariable model		
Factor	**coefficient**	**se**	**p-value**	**coefficient**	**se**	**p-value**
Intercept	*			15.0	5.08	0.005
risk genotypes	8.18	0.74	<0.001	5.90	1.55	<0.001
BMI (kg/m^2)	-0.69	0.22	0.002	-0.45	0.19	0.021
Liver Cirrhosis vs no	6.93	1.91	0.001	5.30	1.65	0.002

Legend: se=standard error. * The estimated intercept's coefficients (se) of univariate models of risk genotypes, BMI and liver cirrhosis were 3.65 (0.74), 23.51 (5.73) and 4.35 (0.78), respectively.

(p=0.019). By contrast, liver cirrhosis was significantly more frequent in subjects with grade 3 than in those with grade 1 and 2 (p=0.001 and p=0.025, respectively). Of the four patients with liver cirrhosis in the lower classes of LIC^{MRI}, three were heavy alcohol drinkers, one had HCV-chronic hepatitis, and all presented with more than two metabolic alterations.

Results of genetic testing according to LIC^{MRI} grading are reported in **Table 2**, showing a higher frequency of genotypes at risk in LIC^{MRI} grade 2 and 3 compared to LIC^{MRI} grade 1 (p=0.003 and p<0.0001, respectively).

Table 3 shows clinical and genetic details of the 13 subjects with major iron overload. A regression analysis identified risk genotypes, liver cirrhosis, and BMI as significantly associated with LIC^{MRI}. **Table 4** reports the coefficients of the univariate and multivariable models. The presence of risk genotypes or liver cirrhosis was associated with an increase in LIC^{MRI} of 5.9 mg/g ($p<0.001$) and 5.3 mg/g ($p=0.002$), respectively. Conversely, for each additional kg/m² in BMI, LIC^{MRI} significantly decreased by 0.45 mg/g ($p=0.021$).

Discussion. In the present study, we showed that: *i.* most of the 66 subjects with BTT and hyperferritinemia were males (89.4%); *ii*. 44% of BTT with hyperferritinemia had normal or slightly increased LIC^{MRI}, another 36% had moderate liver iron overload, while only 20% had LIC above the threshold generally considered at risk for liver damage (7 mg/g); *iii.* The distribution of overweight/obesity, fatty liver, HFE, and non-HFE genotypes favouring iron overload (risk genotypes), and liver cirrhosis significantly differed among the three classes of LIC^{MRI}. The higher prevalence of males is consistent with epidemiological studies showing a high prevalence of hyperferritinemia in men.[25] However, determining the risk of iron-related complications in patients with hyperferritinemia based on serum ferritin levels alone is inadequate, as serum ferritin is an unreliable index of liver iron overload.[16,26,27] In fact, while serum ferritin threshold (> 1000 µg/L) has been established in HFE-hemochromatosis to define such risk,[28] this is a major challenge in other patients with hyperferritinemia, as serum ferritin often overestimates the true amount of LIC.[16,26,27] Accordingly, in this series, serum ferritin levels did not differ between LIC^{MRI} grades 1 and 2, and many patients had levels above 1000 µg/L, even in lower LIC^{MRI} grades (**Figure 1**). Indeed, only a minority of subjects showed LIC^{MRI} above 7 mg/g. This value can be considered a reasonable threshold to distinguish patients at risk of iron-related liver injury who merit iron removal from those who can be followed up to monitor biochemical and clinical trends.[20,21,29] Overweight or obesity and SLD were more frequent in the lower class of LIC^{MRI}. This finding confirms that hyperferritinemia associated with metabolic alterations often overestimates the true amount of liver iron stores, confirming that they both can lead to disproportionate serum ferritin levels. By contrast, liver cirrhosis and risk genotypes were more frequent in the most severe class of LIC^{MRI}. Other factors, such as chronic viral hepatitis, high alcohol intake, and a history of multiple transfusions, might have favoured the development of major iron overload and/or the progression to liver cirrhosis in individual patients. This highlights the need to carefully evaluate hyperferritinemic subjects at the clinical, biochemical, and instrumental levels to ensure adequate clinical monitoring and appropriate therapies.

The role of HFE variants in causing liver iron overload in BTT is controversial, with some suggesting an effect of even single variants in the heterozygous state.[10–14] We have shown that the frequency of heterozygous HFE variants did not differ from that expected in the general population.[30] By contrast, eight out of 13 subjects (61.5%) with severe LIC^{MRI} (**Table 2** and **3**) carried homozygous p.H63D or p.C282Y genotypes, and compound or digenic genotypes (risk genotypes). These results require some further consideration. While it was previously shown that coexistence of BTT can aggravate the iron overload phenotype in subjects homozygous for the p.C282Y mutation,[5] this remains debated for p.H63D homozygosity, which is generally considered a very poor penetrant genotype. However, our findings, together with previous reports,[9,10] support the hypothesis that BTT is an important modifier of p.H63D homozygous penetrance. In the remaining five subjects with high LIC^{MRI}, high alcohol intake, chronic hepatitis, multiple transfusions, and liver cirrhosis were variably present, suggesting they may contribute to the development of iron overload (**Table 3**).[31–35] Multivariable regression analysis showed that overweight, risk genotypes and cirrhosis variably influenced liver iron content as assessed by MRI (**Table 4**). While we can assume that HFE and non-HFE iron-related risk genotypes were causally involved in the development of iron overload, we cannot define the cause-and-effect relationship between iron overload and liver cirrhosis, as iron overload can favour liver fibrogenesis and liver cirrhosis can increase ironabsorption.[29,34] Accordingly, Busti et al.[9] suggested that various cofactors, especially dysmetabolic features, alcohol consumption, and HFE genotypes, can favour the development of hyperferritinemia in BTT, sometimes leading to clinically relevant iron overload.

Conclusions. Our findings indicate that: *i*. even in patients with BTT, the presence of metabolic alterations and excessive alcohol intake should be evaluated before hyperferritinemia is considered a definitive index of major iron overload; these patients should be managed for lifestyle modification to avoid or limit the risk of alcohol- and metabolic-related complications at both hepatic and cardiovascular level and reevaluated at follow-up; *ii*. BTT can be considered a modifier of phenotype expression in individuals with HFE and non-HFE genotypes at risk for iron overload, but it is unlikely that BTT alone or in combination with heterozygous *HFE* variants can cause significant parenchymal iron overload in the liver unless other coexistent factors are present; *iii*. Quantification of liver iron by MRI is a useful tool for distinguishing BTT subjects with hyperferritinemia who can be followed up from those with major iron overload who deserve iron removal

therapies. In clinical practice, accurate collection of patients' medical history (previous transfusions, high alcohol intake, coexistent metabolic and hepatic diseases) and serum iron parameters can be useful to distinguish those who should first be managed with lifestyle changes and clinical-laboratory monitoring from those who should be started directly on MRI quantification of LIC.[16] Iron parameters would guide gene testing,[18] providing further information for the optimal diagnostic and therapeutic approach for patients. Accordingly, in a large cohort of subjects with hyperferritinemia, we recently showed that TSAT and serum ferritin can identify more than 95% of patients with severe LIC, reducing MRI requirements by more than 50%.[15]

Acknowledgements: The project is supported (not financially) by the European Reference Network on Rare Haematological Diseases (ERN-EuroBloodNet)-Project ID No. 10108571. ERN- EuroBloodNet is partly co-funded by the European Union within the framework of the Fourth EU Health Programme. We thank the "Associazione per lo Studio dell'Emocromatosi e delle Malattie da Sovraccarico di Ferro-ETS", Monza, Italy, for supporting the study.

Grant Support: G.R. was funded by the European Union - Next Generation EU - NRRP M6C2 - Investment 2.1 Enhancement and strengthening of biomedical research in the NHS project code PNRR-MAD-2022-12376033, title "Evidence-based models for high impact chronic disease prevention and risk of progression management in outpatient community services and community hospitals: towards eHealth integrating stratification on individual history with predictive models of disease progression, using machine learning and artificial intelligence on administrative and clinical databases", PI Antonio Giampiero Russo. S.G. participated in the manuscript preparation during their personal involvement in the Italian Ministry of University MUR Dipartimenti di Eccellenza 2023-2027 (l. 232/2016, art. 1, commi 314–337). S.G. was partially supported by the grant PRIN 2022SYXEH.

References:

1. Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of β-thalassemia. Eur J Haematol. 2020;105(6):692-703. https://doi.org/10.1111/ejh.13512 PMid:32886826 PMCid:PMC7692954
2. Guimarães JS, Cominal JG, Silva-Pinto AC, Olbina G, Ginzburg YZ, Nandi V, Westerman M, Rivella S, de Souza AM. Altered erythropoiesis and iron metabolism in carriers of thalassemia. Eur J Haematol. 2015;94(6):511-8. https://doi.org/10.1111/ejh.12464 PMid:25307880 PMCid:PMC4393762
3. Pippard MJ, Wainscoat JS. Erythrokinetics and iron status in heterozygous beta thalassaemia, and the effect of interaction with alpha thalassaemia. Br J Haematol. 1987;66(1):123-7. https://doi.org/10.1111/j.1365-2141.1987.00115.x-i1
4. Jones E, Pasricha SR, Allen A, Evans P, Fisher CA, Wray K, Premawardhena A, Bandara D, Perera A, Webster C, Sturges P, Olivieri NF, St Pierre T, Armitage AE, Porter JB, Weatherall DJ, Drakesmith H. Hepcidin is suppressed by erythropoiesis in hemoglobin E β-thalassemia and β-thalassemia trait. Blood. 2015;125(5):873-80. https://doi.org/10.1182/blood-2014-10-606491 PMid:25519750 PMCid:PMC4321326
5. Piperno A, Mariani R, Arosio C, Vergani A, Bosio S, Fargion S, Sampietro M, Girelli D, Fraquelli M, Conte D, Fiorelli G, Camaschella C. Haemochromatosis in patients with beta-thalassaemia trait. Br J Haematol. 2000;111(3):908-14. https://doi.org/10.1046/j.1365-2141.2000.02436.x PMid:11122155
6. 6.Mehta BC, Pandya BG. Iron status of beta thalassemia carriers. Am J Hematol. 1987;24(2):137-41. https://doi.org/10.1002/ajh.2830240204 PMid:3812467
7. Fargion S, Taddei MT, Cappellini MD, Piperno A, Fiorelli G. The iron status of Italian subjects with beta-thalassemia trait. Acta Haematol. 1982;68(2):109-14. https://doi.org/10.1159/000206961 PMid:6812365
8. White JM, Richards R, Jelenski G, Byrne M, Ali M. Iron state in alpha and beta thalassaemia trait. J Clin Pathol. 1986;39(3):256-9. https://doi.org/10.1136/jcp.39.3.256 PMid:3958216 PMCid:PMC499758
9. Busti F, Castagna A, Marchi G, Vianello A, Chesini F, Bozzini C, Lima CDS, Martinelli N, Nemeth E, Ganz T, Girelli D. A case series of patients with β-thalassemia trait and iron overload: from multifactorial hepcidin suppression to treatment with mini-phlebotomies. Haematologica. 2025;110(5):1226-1231. https://doi.org/10.3324/haematol.2024.286413
10. Melis MA, Cau M, Deidda F, Barella S, Cao A, Galanello R. H63D mutation in the HFE gene increases iron overload in beta-thalassemia carriers. Haematologica. 2002;87(3):242-245.
11. Martins R, Picanço I, Fonseca A, Ferreira L, Rodrigues O, Coelho M, Seixas T, Miranda A, Nunes B, Costa L, Romão L, Faustino P. The role of HFE mutations on iron metabolism in beta-thalassemia carriers. J Hum Genet. 2004;49(12):651-655. https://doi.org/10.1007/s10038-004-0202-z PMid:15538648
12. Nadkarni AH, Singh AA, Colaco S, Hariharan P, Colah RB, Ghosh K. Effect of the Hemochromatosis Mutations on Iron Overload among the Indian β Thalassemia Carriers. J Clin Lab Anal. 2017;31(3):e22054. https://doi.org/10.1002/jcla.22054 PMid:27561698 PMCid:PMC6816897
13. Mónaco ME, Alvarez Asensio NS, Haro C, Terán MM, Ledesma Achem ME, Issé BA, Lazarte SS. Effect of HFE Gene Mutations on Iron Metabolism of Beta-Thalassemia Carriers. Thalass. Rep. 2023; 13: 113-121. https://doi.org/10.3390/thalassrep13010010
14. Estevão IF, Peitl Junior P, Bonini-Domingos CR. Serum ferritin and transferrin saturation levels in β0 and β(+) thalassemia patients. Genet Mol Res. 2011;10(2):632-9. https://doi.org/10.4238/vol10-2gmr1016 PMid:21491373
15. Risca G, Pelucchi S, Mariani R, Botti M, Capitoli G, Di Gennaro F, Galimberti S, Piperno A. Transferrin Saturation and Serum Ferritin Are Main Predictors of Liver Iron Content in Subjects With Hyperferritinemia. Clin Gastroenterol Hepatol. 2026;24(1):141-149.e6. https://doi.org/10.1016/j.cgh.2025.02.033 PMid:40398831
16. Piperno A, Pelucchi S, Mariani R. Hereditary Hyperferritinemia. Int J Mol Sci. 2023;24(3):2560. https://doi.org/10.3390/ijms24032560 PMid:36768886 PMCid:PMC9917042
17. Aguilar-Martinez P, Schved JF, Brissot P. The evaluation of hyperferritinemia: an updated strategy based on advances in detecting genetic abnormalities. Am J Gastroenterol. 2005;100(5):1185-94. https://doi.org/10.1111/j.1572-0241.2005.40998.x PMid:15842597
18. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for

the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). J Hepatol. 2024;81(3):492-542.
19. Galimberti S, Trombini P, Bernasconi DP, Redaelli I, Pelucchi S, Bovo G, Di Gennaro F, Zucchini N, Paruccini N, Piperno A. Simultaneous liver iron and fat measures by magnetic resonance imaging in patients with hyperferritinemia. Scand J Gastroenterol. 2015;50(4):429-38. https://doi.org/10.3109/00365521.2014.940380 PMid:25633726
20. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. Blood. 1997 Feb 1;89(3):739-61. Erratum in: Blood 1997;89(7):2621. https://doi.org/10.1182/blood.V89.3.739 PMid:9028304 PMCid:PMC11969382
21. Farmakis D, Porter J, Taher A, Domenica Cappellini M, Angastiniotis M, Eleftheriou A. 2021 Thalassaemia International Federation Guidelines for the Management of Transfusion-dependent Thalassemia. Hemasphere. 2022;6(8):e732. https://doi.org/10.1097/HS9.0000000000000732 PMid:35928543 PMCid:PMC9345633
22. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on haemochromatosis. J Hepatol. 2022;77(2):479-502. https://doi.org/10.1016/j.jhep.2022.03.033 PMid:35662478
23. Girelli D, Busti F, Brissot P, Cabantchik I, Muckenthaler MU, Porto G; on behalf of the Nomenclature Committee of the International Society for the Study of Iron in Biology and Medicine (BIOIRON Society), Hemochromatosis classification: update and recommendations by the BIOIRON Society. Blood 2022; 139 (20): 3018-3029. https://doi.org/10.1182/blood.2021011338 PMid:34601591 PMCid:PMC11022970
24. Tomatsu S, Orii KO, Fleming RE, Holden CC, Waheed A, Britton RS, Gutierrez MA, Velez-Castrillon S, Bacon BR, Sly WS. Contribution of the H63D mutation in HFE to murine hereditary hemochromatosis. Proc Natl Acad Sci USA. 2003;100(26):15788-93. https://doi.org/10.1073/pnas.2237037100 PMid:14673107 PMCid:PMC307646
25. Sandnes M, Ulvik RJ, Vorland M, Reikvam H. Hyperferritinemia-A Clinical Overview. J Clin Med. 2021;10(9):2008. https://doi.org/10.3390/jcm10092008 PMid:34067164 PMCid:PMC8125175
26. Olynyk JK, Gan E, Tan T. Predicting iron overload in hyperferritinemia. Clin Gastroenterol Hepatol. 2009;7(3):359-62. https://doi.org/10.1016/j.cgh.2008.11.010 PMid:19095082
27. Adams PC. The (Il)logic of iron reduction therapy for steatohepatitis. Hepatology. 2015;62(3):668-670. https://doi.org/10.1002/hep.27866 PMid:25914250
28. Allen KJ, Bertalli NA, Osborne NJ, Constantine CC, Delatycki MB, Nisselle AE, Nicoll AJ, Gertig DM, McLaren CE, Giles GG, Hopper JL, Anderson GJ, Olynyk JK, Powell LW, Gurrin LC; Health-Iron Study Investigators. HFE Cys282Tyr homozygotes with serum ferritin concentrations below 1000 microg/L are at low risk of hemochromatosis. Hepatology. 2010;52(3):925-33. https://doi.org/10.1002/hep.23786 PMid:20583211 PMCid:PMC2932765
29. Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. Hepatology. 1986;6(1):24-9. https://doi.org/10.1002/hep.1840060106 PMid:3943787 PMCid:PMC11969382
30. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative haemochromatosis mutations. J Med Genet. 1997;34(4):275-8. https://doi.org/10.1136/jmg.34.4.275 PMid:9138148 PMCid:PMC1050911
31. Harrison-Findik DD. Role of alcohol in the regulation of iron metabolism. World J Gastroenterol. 2007;13(37):4925-30. https://doi.org/10.3748/wjg.v13.i37.4925 PMid:17854133 PMCid:PMC4434614
32. Trinder D, Ayonrinde OT, Olynyk JK. HCV, iron, and oxidative stress: the new choreography of hepcidin. Gastroenterology. 2008;134(1):348-51. https://doi.org/10.1053/j.gastro.2007.11.048 PMid:18166363
33. Shander A, Cappellini MD, Goodnough LT. Iron overload and toxicity: the hidden risk of multiple blood transfusions. Vox Sang. 2009;97(3):185-97. https://doi.org/10.1111/j.1423-0410.2009.01207.x PMid:19663936
34. Ludwig J, Hashimoto E, Porayko MK, Moyer TP, Baldus WP. Hemosiderosis in cirrhosis: a study of 447 native livers. Gastroenterology. 1997;112(3):882-8. https://doi.org/10.1053/gast.1997.v112.pm9041250 PMid:9041250
35. Ioannou GN, Dominitz JA, Weiss NS, Heagerty PJ, Kowdley KV. The effect of alcohol consumption on the prevalence of iron overload, iron deficiency, and iron deficiency anemia. Gastroenterology. 2004;126(5):1293-301. https://doi.org/10.1053/j.gastro.2004.01.020 PMid:15131790