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Consensus statement

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Consensus Statement on the definition and classification of metabolic hyperferritinaemia

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CONSENSUS STATEMENT ON THE DEFINITION AND CLASSIFICATION OF METABOLIC HYPERFERRITINAEMIA

SUPPLEMENTARY DATA

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SUPPLEMENTARY TEXT

SUPPLEMENTARY BOX 1

Noninvasive assessment of tissue iron

Magnetic resonance imaging (MRI) has emerged as the dominant non-invasive imaging modality to quantify hepatic iron content (technically referred to as HIC) [1]. Clinical MRI is based on a characteristic feature of the hydrogen nucleus, consisting of a single proton with a magnetic property called spin, which in specific conditions can absorb energy from radiofrequency pulses. The rate at which the energy absorbed is dissipated to return to the equilibrium once the radiofrequency pulse is interrupted generates a signal, which can be measured to produce an image. The parameters used to describe this process are called relaxation times T1, T2 and T2*. T2 and T2* are of particular interest because they are both decreased in presence of superparamagnetic molecules such as ferritin and haemosiderin, which have a component of iron. As a result, the MRI signal decays faster in iron overloaded organs, and MR images the presence of iron in a tissue indirectly, through its influence on the acceleration of signal decay in surrounding protons [1]. Various MRI techniques have been developed for iron quantification; nowadays the relaxometry technique is considered the standard of care [2,3]. Relaxometry is based on the quantification of relaxation time T2 or T2* in milliseconds, by measuring signal decay at various echo times. Often, the reciprocal of the relaxation times T2 and T2*, R2 and R2* respectively (in Hz) are used (R2 = 1/T2; R2* = 1/T2*). R2 and R2* are both directly proportional to liver iron concentration, whilst T2 and T2* are inversely proportional. Relaxometry techniques are robust, vendor-independent and produce highly accurate non-invasive estimates of hepatic iron. Iron calibration curves are available, correlating tissue iron concentration to liver R2 and R2*[4-7]. In patients with liver disease, various pathologic changes can be present simultaneously, including liver fat, iron, inflammation, biliary disease, and fibrosis [8]. Moreover, the coexistence of several pathologic changes may act as a confounder to liver iron quantification, so a future direction would be the development of multiparametric techniques, to assess each of the pathologic components and control for biologic confounders. Particularly, in patients with fatty liver, the study protocol could include multi-echo chemical shift–encoded gradient-echo sequences for simultaneous assessment of proton-density fat fraction (PDFF) for liver steatosis quantification and T2* (or R2*) for liver iron quantification within a single breath hold [9-11]. PDFF provides validated assessment of liver steatosis [12], and in the end a multiparametric approach could be more cost-effective for the management of complex liver disease.

Unlike conventional treatment strategies, non-invasive imaging diagnosis, therapeutic functions and clinical outcome monitoring could be seamlessly unified in iron theranostics, thereby enabling opportunities to simultaneously quantify and deplete focal iron for dysmetabolic hyperferritinemia. For example, in a proof of principle study clinically used indocyanine green was successfully repurposed as iron theranostics to diagnose and deplete deposited iron in genetic murine models with iron-overload disorders and in a small initial cohort of patients with liver disease [13].

SUPPLEMENTARY BOX 2

Iron accumulation, activation of the BMP-SMAD pathway, modulation of lipid metabolism and cellular damage in hepatocytes

Hepatocytes are central to iron, glucose and lipid metabolism and sustain the interaction of the corresponding metabolic pathways. All these biochemical processes require iron, or its derivatives Fe-S clusters or heme, as cofactors for the function of several key enzymes. For example, iron overload can affect the activity of many hepatic enzymes involved in cholesterol metabolism and can impair desaturation of saturated and essential fatty acids by modulating stearoyl CoA desaturase (SCD) [14]. Iron also regulates glucose homeostasis in both liver and muscle via AMP-activated protein kinase [15], and heme controls the circadian hepatic glucose metabolism in mice [16]. Accumulation of free cytosolic iron leads to oxidative stress, as in mice with *Pcbp1* genetic inactivation in hepatocytes [17]. It also affects mitochondrial function through the generation of reactive oxygen species (ROS) and it increases lipid biosynthetic pathways. Free cytosolic iron activates also ferroptosis, a newly discovered form of regulated cell death characterized by iron-dependent accumulation of lipid peroxides [18,19], that represents the main driver of hepatocyte damage and steatohepatitis in murine NASH [20]. Induction of ferroptosis has been reported to worsen fibrogenesis in models of liver disease [21].

The increased iron level in macrophages in MHF patients may trigger the polarization of these cells towards a pro-inflammatory phenotype [22-24]. Importantly, iron-driven inflammatory phenotypic switching of macrophages promotes fibrosis development [24]. Accordingly, iron deposition in the liver resident macrophages (Kupffer cells) has been associated with NASH and advanced fibrosis in patients with NAFLD [25], while mice on low iron are protected from NASH [26]. It should however be mentioned that, although severe suppression of the BMP-SMAD pathway and hepcidin release lead to severe iron overload and fibrosis [27], activation of BMP-SMAD by hepatic iron accumulation ameliorated steatosis in mice due to increased lipolysis and downregulation of PPARγ signaling [28].

SUPPLEMENTARY BOX 3

Iron and vascular damage

Epidemiological studies suggest that dietary intake of heme iron is associated with an increased risk of cardiovascular disease [29]. Iron retention in macrophages as well as vascular cells might play a role also in the development of cardiovascular disease in MHF through the formation of foam cells, alterations of the vascular walls, and vascular stiffness [30,31]. Patients with metabolic alterations often show hypertension and increased arterial stiffness, which predispose to cardiovascular disease. Interestingly, hyperferritinemia is associated with aggravated aortic stiffness and cardiac diastolic dysfunction [32,33], suggesting an interaction between iron dys-homeostasis and altered cardiovascular functions. Interestingly, serum ferritin has been described as a potentially imprinting factor given its association with carotid intima media thickness in offspring of fathers (but not mothers) with increased serum ferritin levels [34]. Overall, iron overload increases the vasoconstrictor response of arteries, associated with altered vascular reactivity and the loss of endothelial modulation of the vascular tone [30,35]. Hepcidin-induced iron accumulation has been shown to drive vascular smooth muscle cell proliferation, contributing to vascular remodeling and pulmonary hypertension [30,36]. In addition, hepcidin-mediated intracellular iron trapping in macrophages results in exacerbated inflammation and impaired cell cholesterol handling, due to enhanced CD36-mediated cholesterol uptake and decreased ABCA1/ABCG1-mediated reverse cholesterol efflux. These mechanisms likely promote foam cells formation within vascular lesions and plaque destabilization through inflammatory cytokine release, intracellular lipid loading, oxidative stress, and cell apoptosis [30,37-39]. Accordingly, hyperferritinemia, hepcidin levels and macrophage iron positively correlate with IL-6 and MCP-1 levels and the presence of carotid plaques in individuals with metabolic syndrome and NAFLD [40,41].

SUPPLEMENTARY TABLES

Supplementary Table 1. Main recommendations presented in the manuscript and consensus reached among authors.

#	Recommendation	Consensus	*Total*	*Partial*	*Neutral*	*Limited*	*No*
1.	Insulin resistance and features of the metabolic dysfunction are associated with specific alterations of iron metabolism regulation, which are epidemiologically linked with organ damage and clinical outcomes.	**100%**	**80**	**17**	**3**		
2.	The pathophysiology of this alteration of iron metabolism regulation seems triggered by lipotoxicity in the presence of permissive environmental and genetic determinants, but additional studies are required to clarify the contribution of subclinical inflammation and the underlying mechanisms and implications.	**100%**	**71**	**26**	**3**		
3.	We propose as the most accurate and available biomarker to non-invasively capture and grade the presence of the aforementioned iron metabolism alteration, associated with glucose and lipid metabolism dysregulation and with hepatic fat accumulation, serum ferritin (SF).	**97%**	**68**	**26**	**3**	**3**	
4.	We propose to define this condition as "Metabolic hyperferritinemia" (MHF), and to grade its severity according to SF levels thresholds (grade 1 to 3), which will need prospective validation and optimization. When possible, SF levels should be evaluated after at least 3 months of lifestyle changes.	**100%**	**83**	**17**			
5.	As criteria for "Metabolic dysfunction", we propose those matching the definition of "Metabolic dysfunction associated fatty liver disease" (MAFLD), with the following modifications: inclusion of the presence of fatty liver among the criteria, exclusion of those with biochemical signs of overt inflammation and of heavy alcohol intake.	**93%**	**90**	**3**		**7**	
6.	Given the initial evidence that in stable patients with MHF, SF may be associated with tissue iron accumulation, we suggest to non-invasively estimate hepatic iron concentration by MRI in clinical studies. This can be considered, when available, in pathophysiological studies and in clinical practice in patients with higher SF (grade 2, but in particular grade 3) and/or additional clinical risk factors for iron overload. When available, tissue iron concentration should have the priority on SF to grade MHF.	**97%**	**74**	**23**		**3**	
7.	We propose to define the presence of "Dysmetabolic iron overload syndrome" (DIOS) in patients with MHF and increased hepatic iron stores, as evaluated by R2*>140 1/s or direct evidence of increased hepatic iron stores.	**100%**	**74**	**26**			
8.	Liver biopsy is not required for the diagnosis of MHF and of DIOS, unless otherwise indicated for the management of associated liver disease or for specific research purposes.	**100%**	**93**	**7**			
9.	The clinical management should be focused on the correction of overweight and lifestyle factors associated with increased risk of cardiometabolic risk factors (e.g. dietary caloric intake and pattern, alcohol, fructose and salt intake, sedentary lifestyle) and the pharmacological control of cardiovascular risk factors.	**100%**	**93**	**7**			
10.	In patients with MHF and DIOS, iron depletion (ID) therapy should be considered as an experimental therapy to be tested in well-powered controlled trials.	**100%**	**70**	**23**	**7**		
11.	Additional studies are required to define the specific genetic and environmental risk factors for MHF and DIOS development.	**100%**	**100**				
12.	Blood donation is not contraindicated in individuals with MHF with controlled cardiovascular risk factors, in the absence of organ damage and of other contraindications to phlebotomy.	**100%**	**93**	**7**			
13.	Additional studies are required to define the correlation between SF levels and hepatic iron content determined by MRI in patients with MHF.	**100%**	**87**	**10**	**3**		
14.	Additional studies are required to investigate whether MHF and/or mild tissue iron accumulation in the liver, adipose tissue and other organs are causally involved in the pathogenesis of insulin resistance, liver disease and other chronic degenerative conditions associated with MHF.	**100%**	**93**	**7**			
15.	Clinical studies evaluating ID in MHF/DIOS patients should consider as main outcomes biomarkers more closely linked to clinical events and take into account the perceived quality of life, which should be assessed after an adequate duration of follow-up, at least 3 months after ID achievement in the active arm.	**97%**	**61**	**33**	**3**	**3**	

Supplementary Table 2. Differential diagnosis of metabolic hyperferritinemia (MHF).

COMMON DISORDERS	
Inflammatory disorders	Infections, including viral infections (e.g. COVID-19).
	Sepsis, SIRS, MOF, MAS.
	Connective tissue and other autoimmune disorders, including inflammatory arthritis.
	End stage renal disease and dialysis.
Alcohol abuse	Alcoholic steatohepatitis, chronic alcohol use disorder.
Liver disease	Acute and chronic liver damage (acute and acute on chronic liver failure, acute and chronic hepatitis).
Neoplasia	Lymphoproliferative disorders, some forms of solid cancer, disseminated neoplasia.
Myelodysplastic syndromes (MDS)	Hyperferritinemia due to ineffective erythropoiesis, RBC transfusions, and/or blast-derived inflammatory cytokines [42]. Refractory anemia with ring sideroblasts (RARS) is the MDS-form with the highest values of iron parameters before the onset of transfusion therapy.
Hemolytic disorders	Acquired hemolytic anemias.
RARE AND GENETIC DISORDERS	
	Hemochromatosis and other iron metabolism disorders (e.g. Ferroportin disease, aceruloplasminemia).
	Thalassemias, dyserythropoietic, sideroblastic and hemolytic anemias and other RBC disorders (e.g. RBC membrane and enzymatic defects) [43].
	Lysosomal storage disorders (e.g. Gaucher disease) [44,45].
	Hereditary hyperferritinemia with or without cataract [46].

SIRS: systemic inflammatory response syndrome; MOF: multiorgan failure; MAS: macrophage activation syndrome, RBC: red blood cells.

Supplementary Table 3. Proposed clinical assessment in patients with metabolic hyperferritinemia (MHF; for clinical and research purposes). These may not be indicated for all patients in clinical practice but can be considered based on the individual history and presentation features.

CLINICAL	
Clinical history	Family history of iron metabolism, erythropoiesis, cardiometabolic, neoplastic and liver disorders; history of metabolic, erythropoiesis, neoplastic, liver and inflammatory disorders, drugs, iron supplementation, RBC transfusion.
Lifestyle factors	Alcohol intake (amount and pattern), physical activity, dietary pattern, use of dietary supplements, environmental exposures.
Anthropometric parameters	Height, weight, BMI, abdominal circumference.
BIOCHEMICAL	
Iron status	Serum iron, transferrin, transferrin saturation %, ferritin.
Cofactors	Ceruloplasmin levels, electrophoresis.
Inflammation	CRP, autoimmunity markers if strong clinical suspicion.
Red blood cells	Complete blood count with formula; LDH, bilirubin, haptoglobin and reticulocytes if clinical suspicion of hemolysis; hemoglobin electrophoresis if clinical suspicion of beta-thalassemia or other hemoglobinopathies carriage; blood smear, hemolysis markers and/or hematological referral if clinical suspicion of RBC defects, dyserythropoietic or sideroblastic anemias, or myelodysplastic syndromes [47].
Liver damage	AST, ALT, GGT, FIB-4 score.
CANCER SCREENING	
	According to screening guidelines, but keep higher index of suspicion, rule out in the presence of risk factors.
IMAGING	
Upper abdominal ultrasound	To evaluate fatty liver, signs of liver disease and spleen size.
Transient elastography (e.g. Fibroscan)	To screen for fatty liver by CAP, to stage liver disease severity by LSM when FIB-4>1.3, to monitor steatosis and liver damage.
Abdominal MRI	To estimate hepatic iron content (by R2*; possibly converted to μmol/g); association with cardiac or spleen iron presently unknown. If available, simultaneous assessment of proton-density fat fraction (PDFF) for liver steatosis.
LIVER BIOPSY	
	To rule out NASH and stage liver damage for clinical trials; in the presence of liver damage more severe than expected to rule out concurrent etiology; to stage fibrosis (advanced fibrosis) when non-invasive assessment is indeterminate for HCC surveillance.
GENETICS	
Common *HFE* variants*	Family history and/or persistently increased TS% especially in Caucasian patients [48].
Rare *HFE* variants and other hemochromatosis genes variants	Family history and/or persistently increased TS% especially in juvenile or severe phenotypes, non-Caucasian patients, or in the absence of *HFE* common variants [48].
SERPINA1 variants	Family history, altered alpha-1 fraction at electrophoresis or reduced circulating A1AT.
CERULOPLASMIN variants	Reduced circulating CP, family history, low transferrin saturation with severe stores, and the possible presence of anemia, microcytosis, diabetes

	and neurologic complications.
FPN1 variants	Family history of iron overload (AD).
Iron genes panel**	Severe iron accumulation (Grade 3 MHF, DIOS) [49,50].

Abbreviations: AD: autosomal dominant; AAT: alpha1-antitrypsin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; CAP: continuous attenuation parameter; CP: ceruloplasmin; GGT: gamma-glutamyltransferase; HCC: hepatocellular carcinoma; LSM: liver stiffness measurement; MRI: magnetic resonance imaging; NASH: nonalcoholic steatohepatitis; TS%: percentage of transferrin saturation.

* In spite of the reported associations of *HFE* variants with increased ferritin and progression to DIOS, currently there is no demonstration of clinical utility of HFE genotyping in these patients because by itself it is not sufficient to identify patients with excessive hepatocellular iron.

**When available and / or for research purposes; demonstration of increased tissue body iron stored is advised before evaluation of genetic predisposition outside the research setting.

Supplementary Table 4. Clinical correlates of ferritin levels and hepatic iron stores in individuals with metabolic dysfunction.

Outcomes	*Main results*
TYPE 2 DIABETES	Hepatic iron and ferritin associated with more severe insulin resistance and metabolic syndrome [40,51-56]
	Ferritin predicts higher incidence of type 2 diabetes [57-59]
	Ferritin and iron supplementation predict gestational diabetes mellitus [60-62]
CARDIOVASCULAR DISEASE	Ferritin associated with carotid damage and vascular stiffness [32,33,40]
	Hepcidin associated with inflammation and vascular damage [41]
LIVER DISEASE	Hepatic siderosis (predominantly non-parenchymal or mixed) associated with nonalcoholic steatohepatitis (NASH) and fibrosis in patients with nonalcoholic fatty liver disease (NAFLD) in some studies [63-65]
	Genetic variants favoring hepatic iron deposition associated with liver fibrosis, cirrhosis and hepatocellular carcinoma [49,66]
	Genetically predicted increase in liver iron level associated with an increased risk of MAFLD (Mendelian randomization approach to assess causality) [67]
	Hepcidin associated with liver damage and hyperferritinemia [68]
	Liver iron (non-parenchymal or mixed) associated with hepatocellular carcinoma [69]
	Liver iron associated with worse clinical hepatic and cardiovascular outcomes in nonalcoholic fatty liver disease [70]
KIDNEY DISEASE	Ferritin and hepatic iron associated with microalbuminuria and kidney disease in type 2 diabetes [71,72]
MORTALITY	Higher overall and cardiovascular mortality in men without severe chronic diseases [73]

Supplementry Table 5. Main controlled studies reporting the impact of iron depletion by phlebotomy in individuals with metabolic hyperferritinemia (MHF).

Study	*Design*	*Sample*	*Follow-up*	*Outcome*	*Notes*
Fernández-Real et al. [74]	RCT 1:1 to ID or control	28 patients with T2D and hyperferritinemia	12 months	Reduced HbA1c and insulin resistance, improved insulin secretion	ID by 3 phlebotomies
Valenti et al. [53]	Case-control study	128 patients with NAFLD	6-24 months	Reduced insulin resistance in those with ferritin >250 ng/ml	
Zacharski et al. [75]	RCT: 1:1 to ID or control	1231 patients with peripheral arterial disease	6 years	Reduced cancer incidence	Secondary analysis of a RCT
Valenti et al. [76]	PS-matched analysis	198 patients with NAFLD	6-8 months after ID achievement	Reduced insulin resistance and ALT, more frequent ALT normalization	
Valenti et al. [77]	RCT: 1:1 to ID or lifestyle changes alone	35 patients with NAFLD and hyperferritinemia	24 months	Reduced AST, ALT, GGT; weight gain; improved liver histology	
Adams et al. [78]	RCT 1:1 to ID or lifestyle	74 patients with NAFLD irrespective of ferritin	6 months	No effect on insulin resistance or ALT	
Laine et al. [79]	RCT 1:1 to ID or lifestyle changes	274 patients with DIOS	12 months	Lack of improvement in insulin resistance; weight gain; reduced ALT	DIOS determined by MRI; insulin resistance main outcome
Mateo-Gallego et al. [80]	RCT 1:1 to 3 phlebotomies or control	86 patients with hyperferritinemia and hyper-TG	9 weeks	Lack of improvement in TG; improvement in RBP-4	Trend for improvement in liver enzymes, larger impact in those who reduced iron stores

RCT: randomized controlled trial; ID: iron depletion by phlebotomy; NAFLD: nonalcoholic fatty liver disease; PS: propensity score; ALT: alanine aminotransferases; DIOS; dysmetabolic

iron overload syndrome; MRI: magnetic resonance imaging; TG: circulating triglycerides; RBP-4: retinol binding protein-4.

Supplementary Table 6. Main research challenges in the metabolic hyperferritinemia (MHF) field.

	Goal	*Notes on design*
DIAGNOSIS	Validate the updated definition. Validate suggested serum ferritin cut-offs.	Examine the added clinical value (for risk stratification of clinical events and prediction of response to therapy).
	Validate non-invasive assessment of iron accumulation and staging.	Validation of ferritin levels against tissue iron concentration and clinical outcomes.
EPIDEMIOLOGY	Re-assess the prevalence in different ethnic groups and clinical settings.	With staging of severity. Assessment of SF reduction after at least 3 months of standardized lifestyle change.
	Evaluate the interaction between dysmetabolism/IR and alcohol intake in the pathogenesis of MHF.	Consider also other dietary factors.
	Refine risk factors and develop polygenic risk scores.	Polygenic risk scores may be used in Mendelian randomization studies to estimate the causal role in determining organ-specific complications in observational studies in large cohorts.
PATHOPHYSIOLOGY	Identify additional risk loci to improve risk stratification, disease knowledge and identify new therapeutic targets.	
	Identify at-risk/high-risk phenotypes for carriage of variants of iron genes	
	Clarify the pathophysiological mechanisms and biological pathways linking iron and lipid/glucose metabolism.	
	Clarify the relationship between ferritin levels and markers of subclinical inflammation.	
	Investigate the possible role of hyperferritinemia per se in disease progression (immunological properties of ferritin, its role as pro-inflammatory signaling molecule and iron delivery system, e.g. as iron source and pro-oxidant activity).	
CLINICAL ASSESSMENT	Clarify the specific contribution of deranged iron metabolism vs. tissue-specific accumulation in the pathogenesis of disease outcomes.	Examine role of iron vs. fat accumulation in the liver, adipose tissue, skeletal muscle, spleen, pancreas.
	Standardize clinical assessment and organ damage staging.	It will require demonstration of an impact on the ability to stratify the risk of outcomes.
	Evaluate the diagnostic yield of targeted sequencing of iron metabolism gene panels or other next generation sequencing approaches in patients with DIOS.	It will have clinical implications for the management of patients with DIOS.
TREATMENT	Evaluate the long-term impact of iron depletion (and more generally of modulation of iron status) on metabolic, cardiovascular, neoplastic and hepatic outcomes in well-powered long-term studies.	Design randomized controlled trials stratified for MHF severity (Baseline SF and hepatic iron content) to define the optimal threshold for which iron depletion may be potentially beneficial; Consider stratification for genetic risk factors; Assess after at least 3 months of lifestyle change; Design trials testing the effects of sustained modification of diet and lifestyle habits on ferritin levels and iron stores; Standardize iron depletion protocols and maintenance, and possible support therapies (e.g. folate supplementation); Identify iron depletion/ modulation protocols not affecting the patient's functional capacity; Examine outcomes at least at 3 months after normalization of iron stores; Consider observational studies in blood donors controlling for donation frequency and propensity score; Consider as outcomes incidence of diabetes, major cardiovascular events (markers of atherosclerosis), cancer, liver events (progression of liver fibrosis), patient perceived quality of life.

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