Consensus statement on the definition and classification of metabolic hyperferritinaemia

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Abstract

Hyperferritinaemia is a common laboratory finding that is often associated with metabolic dysfunction and fatty liver. Metabolic hyperferritinaemia reflects alterations in iron metabolism that facilitate iron accumulation in the body and is associated with an increased risk of cardiometabolic and liver diseases. Genetic variants modulating iron homeostasis and tissue levels of iron are the main determinants of serum levels of ferritin in individuals with metabolic dysfunction, raising the hypothesis that iron accumulation might be implicated in the pathogenesis of insulin resistance and the related organ damage. However, validated criteria for the non-invasive diagnosis of metabolic hyperferritinaemia and the staging of iron overload are still lacking, and there is no clear evidence of a benefit for iron depletion therapy.

Here, we provide an overview of the literature on the relationship between hyperferritinaemia and iron accumulation in individuals with metabolic dysfunction, and on the associated clinical outcomes. We propose an updated definition and a provisional staging system for metabolic hyperferritinaemia, which has been agreed on by a multidisciplinary global panel of expert researchers. The goal is to foster studies into the epidemiology, genetics, pathophysiology, clinical relevance and treatment of metabolic hyperferritinaemia, for which we provide suggestions on the main unmet needs, optimal design and clinically relevant outcomes.

[H1] Introduction

Ferritin is the main intracellular iron storage protein and is a biomarker of iron stores and inflammation. An increased serum concentration of ferritin is a common biochemical finding, with a prevalence of 5.9–19.0% in apparently healthy individuals, depending on ethnicity [1]. As ferritin is the main protein responsible for iron storage in the body, it represents a valuable biomarker of total body iron in iron overload conditions such as haemochromatosis, where it is used to guide iron-reduction therapy [1]. Ferritin is also an acute phase reactant, and levels of ferritin are often increased in chronic inflammatory conditions, such as in individuals with insulin resistance and metabolic dysfunction (that is, the presence of metabolic alterations, including type 2 diabetes mellitus and/or obesity or at least two features typically associated with insulin resistance (increased visceral adiposity, atherogenic dyslipidaemia, arterial hypertension, hyperinsulinaemia)) [2]. Serum levels of ferritin have been associated with the amount of hepatic lipid accumulation, the severity of insulin resistance and features of metabolic dysfunction, even in the absence of inflammation [3-13]. Insulin resistance and metabolic dysfunction have also been linked to an iron overload syndrome featuring hyperferritinaemia and accumulation of iron predominantly in non-parenchymal liver cells (Kupffer cells); this syndrome shares epidemiological, genetic and biochemical features with hyperferritinaemia related to metabolic dysfunction [14]. This condition, featuring hyperferritinaemia related to metabolic dysfunction, was initially named insulin resistance-associated hepatic iron overload syndrome, and subsequently defined as dysmetabolic iron overload syndrome (DIOS) or dysmetabolic hyperferritinaemia [14-17]. Unlike patients with haemochromatosis, patients with DIOS have fairly preserved production of hepcidin, the iron regulatory hormone secreted by the liver in response to iron and inflammation [14-16]. The

coexistence of different definitions (with some of them, e.g. DIOS, requiring the histological demonstration of non-parenchymal tissue iron overload and others not, and using different criteria to define metabolic dysfunction) has so far posed severe limitations on the comparability of the results and conclusions of studies published in this field.

Individuals with hyperferritinaemia related to metabolic dysfunction, that here we named "metabolic hyperferritinaemia" (MHF) show a variable degree of iron stores in the body, which are partly correlated with serum levels of ferritin [14]. In individuals with MHF, iron stores in the body range from normal to a moderate iron overload, usually below levels reached in haemochromatosis [14]. Indeed, elevated serum levels of ferritin can develop in individuals with metabolic alterations in the presence of risk factors for iron accumulation (for example, genetic risk variants, male sex and older age and are associated with normal transferrin saturation [14]. Therefore, the spectrum of iron metabolism alterations in individuals with MHF ranges from minor biochemical changes to tissue iron accumulation, which might lead to iron-related organ damage [14].

Previous categorization attempts focused on the most severe forms of MHF, such as DIOS where hyperferritinaemia is also associated with tissue iron overload, and were based on heterogeneous and unclear criteria to define iron accumulation as related to metabolic dysfunction. Historically, the diagnosis DIOS was based on histological demonstration of iron accumulation in Kupffer cells [14-16]. Liver histology has been pivotal to support the diagnosis of this condition in patients with clinically relevant iron overload. However, given its invasive nature, liver biopsy has limited the assessment of iron overload in clinical practice and in research studies. Progress in imaging techniques now enables the accurate noninvasive estimation of tissue concentrations of iron by MRI (Supplementary Box 1). MRI can approximate the cellular predominance of iron accumulation by assessing levels of iron in the spleen and liver. Accumulating evidence suggests that the hepatic and total body content of iron in patients with metabolic dysfunction correlates with serum levels of ferritin [6,7,18-22]. Importantly, most, but not all, studies [23] have shown that in patients with marked hyperferritinaemia despite the absence of acute inflammation, a considerable increase in hepatic and body iron stores is typically seen [19,24]. However, the amount of iron accumulation rarely reaches thresholds defining haemochromatosis and other primary iron disorders, where excess iron is sufficient to drive progressive organ damage [6,7,18-21]. The pattern of iron

overload is also distinct in DIOS and haemochromatosis, with iron overload predominantly in macrophages being typical of DIOS [25].

[H1] Methodology

Given the high prevalence of hyperferritinaemia in clinical practice, combined with a growing understanding of the underlying pathophysiology and clinical implications, an updated definition and grading system is warranted. The overall goal of this Consensus statement was therefore to provide a proposal for a more accurate diagnosis and classification of MHF, to be validated by prospective studies, to provide suggestions on the design of these studies and to highlight some of the main unmet research needs in the field. Due to the current absence of robust evidence supporting the recommendation to screen for and diagnose MHF and then to treat this condition with a specific approach (such as iron depletion), the current Consensus statement is mainly directed at clinicians working in tertiary referral centres and clinical and basic researchers. The updated MHF definition will enable larger collaborative studies on the clinical implications, pathophysiology and therapy of this condition, although it will still require prospective validation and further refinement. The long-term goal is the improvement of clinical management of patients with MHF. Based on the current evidence, we also provided expert recommendations for clinicians on the diagnosis, management, follow-up and treatment of this condition (presented in the supplementary material).

Consensus was reached by a multidisciplinary global panel of expert researchers with an interest in MHF from five continents and 14 countries working in the fields of iron metabolism, clinical endocrinology, hepatology, radiology and haematology. The panel was selected to include the main research groups active in the field, and to cover all of the main disciplines involved and the different geographical areas. We considered corresponding authors of papers related to MHF and representatives of the main regional scientific societies. The initial 15 statements (Supplementary Table 1) were drafted by the two corresponding authors after an initial review of the literature; the corresponding authors drafted the results of this literature review, developed written proposals and wrote the first manuscript draft. Statements relevant for the clinicians were marked as ‘Clinically relevant’ (CR). Written feedback was received from all the authors and this feedback was discussed through personal meetings, online teleconferences and e-mails during the COVID-19 pandemic.

Consensus on the 15 recommendations was subsequently tested formally by means of an initial Delphi procedure conducted by online questionnaires on June 1st, 2022. The data collection period ranged for 4 weeks. The round 1 survey contained five domains with five-point Likert-type categories for respondents to indicate their level of agreement with the statements (that is, 'Agree', 'Somewhat agree', 'Neither agree nor disagree', 'Somewhat disagree' or 'Disagree'). In the first round, respondents who agreed or somewhat agreed with a statement could provide comments or suggest edits, while those who disagreed or somewhat disagreed needed to explain why. Results are reported in detail in Supplementary Table 1. Agreement by ≥75% of participants was required to enable a recommendation, which was achieved for all proposals during the first Delphi round. However, further discussion was undertaken by email to report the results of round 1 and the comments and disagreements in round 1, which were taken into consideration in the final version of the manuscript. For the Delphi process, we assigned each statement and recommendation a grade to indicate the level of agreement utilising the grading system used in other Delphi studies, in which 'U' denotes unanimous (100%) agreement, 'A' 90–99% agreement, 'B' 78–89% agreement and 'C' 67–77% agreement [26].

The study was designed, and the recommendations finalized by L.V. and E.C.. The first manuscript draft was written by: L.V., E.C., D.Mc., B.H., A.Pa., D.P., L.S. and F.V., and was reviewed for important intellectual content by L.A.A., H.H., S.A., E.B., E.A.T., J.-M.F.-R., J.D.R., P.P., and H.Z.. All authors developed the recommendations, reviewed the first manuscript draft, participated in the Delphi consensus process, reviewed and approved the final manuscript.

[H1] Recommendations

[H2] Epidemiology and risk factors

R1. 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 (U).

Not all patients with metabolic dysfunction or fatty liver present with increased serum levels of ferritin, suggesting that those with MHF constitute a subset with distinct risk factors, pathophysiology and clinical outcomes, possibly deserving specific management. **[Au: Tables have to have more than two columns, so I have changed table 1 to a box. I'll renumber the display items once you return the revised text.]** Box A outlines the main genetic and acquired conditions associated with hyperferritinaemia and accumulation of iron in the body in individuals with metabolic dysfunction, fatty liver and insulin resistance.

Serum levels of ferritin were first described to be linked to insulin resistance in 1998 [27]. The severity of insulin resistance [3-11,28] and the severity of fatty liver disease, but not circulating biomarkers of inflammation, have been reported as determinants of MHF [6,7,18-21,29,30]. By contrast, genetic variants associated with iron metabolism, rather than those affecting lipid handling in the liver, were associated with increased serum levels of ferritin and MHF in patients with fatty liver [31]. This observation is in line with findings indicating that serum levels of ferritin reflect hepatic and body iron stores more closely than the severity of liver lipid accumulation in individuals with metabolic dysfunction [6,7,18-21]. Furthermore, male sex, older age and moderate alcohol intake contribute to iron accumulation in fatty liver disease. This evidence is based on liver histology and MRI evaluations of cohorts of patients with fatty liver or multiple metabolic alterations [6,7,18-21]. Therefore, in patients with insulin resistance but without acute inflammatory conditions, severe alcohol abuse or poorly controlled diabetes mellitus, serum levels of ferritin are mainly determined by a dysregulation of iron metabolism, and next by the severity of insulin resistance.

In a large prospective cohort of middle-age healthy men conducted in South Korea, elevated serum levels of ferritin were independently associated with development of the metabolic syndrome during the 5-year follow-up period [32]. Furthermore, in a large prospective study on European cases of incident type 2 diabetes mellitus, increased serum levels of ferritin were associated with an increased risk of type 2 diabetes mellitus, even among individuals with no overt inflammation, liver disease, high alcohol consumption or obesity [33].

Data from the past 25 years in European populations suggest that serum concentrations of ferritin are associated with insulin resistance and metabolic dysfunction, even in individuals without fatty liver or with liver levels of iron within the reference range [3-11,27]. However, in individuals with high serum concentrations of ferritin, the presence of fatty

liver might indicate an increased risk of insulin resistance and the metabolic syndrome, whereas the presence of hepatic iron overload might indicate an increased risk of hyperglycaemia [34]. These data are in line with genetic evidence from the past decade that body stores of iron have a causal role in determining liver disease and the development of type 2 diabetes mellitus in the general population [35-37].

By means of Mendelian randomization as a robust epidemiological approach to analyze the causal estimation of fatty liver disease associated with metabolic dysfunction (also known as MAFLD) as an outcome using genetic variants, a study published in 2022 that included European individuals showed that the genetically predicted increase in liver levels of iron was associated with an increased risk of fatty liver disease. Although they need confirmation, these data support a causal association between deposition levels of iron in the liver and metabolic dysfunction [38].

[H2] Pathogenesis

R2. The pathophysiology of this alteration of iron metabolism regulation seems to be 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 (U).

[H3] Mechanism of iron accumulation. The pathogenesis of MHF is multifaceted, relating to the effect of common genetic variants on iron homeostasis, as well as hepatic, intestinal and adipose tissue factors. Systemic iron homeostasis is maintained through the hepcidin–ferroportin axis. Hepcidin is a liver peptide hormone whose expression is upregulated by iron and inflammation. Hepcidin controls iron influx into the blood stream from duodenal enterocytes and macrophages by binding, occluding and inducing the degradation of the iron exporter ferroportin. As a consequence of the increased hepcidin secretion, iron accumulates in cells expressing ferroportin, mainly macrophages and, to a lesser extent, hepatocytes. Genetic iron overload disorders (such as haemochromatosis) are most frequently caused by reduced hepcidin synthesis or impaired iron export [39]. In patients with MHF, hepcidin release in response to iron stores and the ability of hepcidin to downregulate intestinal iron

absorption are generally preserved [40]. However, a subtle alteration in iron fluxes has been reported, whereby excess fatty acids have been linked to a reduced ability of hepcidin to limit intestinal iron absorption, while simultaneously increasing hepatic iron uptake and tissue deposition [40-43].

Interestingly, patients with type 2 diabetes mellitus without clinical signs of inflammation show MHF with reduced hepcidin levels and increased systemic levels of iron [44]. Furthermore, the development of type 2 diabetes mellitus might be associated with impaired hepcidin release induced by hyperinsulinaemia [45]. In patients with metabolic dysfunction and hyperferritinaemia, body stores of iron have been associated with high dietary iron intake [46] and a distinct microbiome profile [47]. In patients with MHF or obesity, development of mild iron accumulation in hepatocytes can lead to hepcidin upregulation and restrains further iron absorption [48-50]. Preserved regulation of hepcidin would favour the preferential retention of iron in liver macrophages (Kupffer cells), where ferroportin is highly expressed [31,51,52]. Within this context, the presence of inherited variants associated with impaired hepcidin would favour the development of more severe iron accumulation and DIOS (Table 1).

Reduced expression of the ferroxidase ceruloplasmin, which cooperates with ferroportin for iron export in several cell lineages, including hepatocytes, might also favour iron accumulation in the liver. Furthermore, low-frequency inherited genetic variants of *CP* gene (which encodes ceruloplasmin) that determine a mild functional impairment of ceruloplasmin were associated with MHF and more severe liver disease in patients with fatty liver disease [31].

[H3] Role of excess iron in tissue damage and insulin resistance. While the role of iron overload in determining liver disease (liver fibrosis progression and hepatocellular carcinoma) and pancreatic β-cell failure is established in haemochromatosis [39], the potential effect of dysregulation of iron metabolism on the pathogenesis of dyslipidaemia and insulin resistance is less clear. A detailed discussion of the complex relationship among iron accumulation, activation of the bone-morphogenic protein (BMP and SMAD) signalling pathways, modulation of lipid metabolism and development of liver disease is presented in Supplementary Box 2. This process might involve the facilitation of ferroptosis as well as of other forms of cell death in hepatocytes and other liver cells [53]. Accumulation of iron in macrophages in the liver has been associated with more severe liver damage in patients with

fatty liver disease [20,54], compared with patients without iron accumulation in these cells. By catalyzing the formation of reactive oxygen species (ROS), excess iron favours subclinical inflammation, which contributes to insulin resistance by directly downregulating insulin receptor expression and signalling and by worsening of alterations of glucose and lipid metabolism [10,44,55,56], fibrogenesis and carcinogenesis [14]. Unlike in patients with haemochromatosis, who are typically iron depleted because of hepcidin deficiency, iron accumulation in macrophages and hepatic stellate cells has been associated with a pro-inflammatory and pro-fibrotic response [57,58]. Moreover, excess fatty acids and lipotoxicity predispose to inflammation and type 2 diabetes mellitus by inducing macrophage iron accumulation via induction of *FTH1*, which encodes the ferritin H subunit (which has iron oxidase activity) [59].

In addition to insulin resistance and dyslipidaemia, ferritin levels and levels of iron stores in the liver have also been associated with the build-up of iron in adipose tissue, which leads to insulin resistance, impaired adiponectin secretion and altered endocrine function in murine models and in patients [55,56,60], as well as impaired regulation of amino acid and phospholipid metabolism in patients with fatty liver [61,62]. Indeed, ferroportin is also expressed in adipocytes, where it can be targeted by hepcidin to exert its endocrine function [55]. Insulin resistance and the expansion of adipose tissue after overfeeding modulate the expression of iron-related genes, such as transferrin receptor (*TFRC*) and *FTH1* in adipose tissue in patients with metabolic alterations [63,64]. Increased iron levels in adipocytes, such as in conditions of high levels of hepcidin or adipose tissue specific *FPN* deletion in experimental models, negatively regulates expression of the genes that encode leptin and adiponectin [55,65]. This mechanism might contribute to the development of obesity and insulin resistance. The crucial role of adipose levels of iron in the development of insulin resistance was supported by evidence demonstrating that adipose-specific genetic variants that decreased expression of the iron export protein ferroportin led to insulin resistance and obesity [66]. Although the exact mechanism remains to be elucidated, paracrine trafficking of iron between adipocytes and macrophages in adipose tissue is altered by high-fat diet feeding in mice, which contributes to changes in macrophage polarization [57,67]. Iron accumulation might also be directly involved in facilitating vascular damage, including by epigenetic mechanisms (Supplementary Box 3).

In line with the concept of a multi-level crosstalk between the adipose tissue and the gut in controlling caloric and nutrient influx, low iron levels in adipose tissue, as a result of

constitutive or inducible adipocyte-specific transferrin receptor 1 (*TFRC*) deficiency or AAV-mediated overexpression of the iron exporter ferroportin in mature adipocytes, protects mice from high-fat-diet-induced metabolic disorders [68]. In such preclinical models, reduced cellular levels of iron in adipocytes have been associated with the restriction of lipid absorption from enterocytes following high-fat diet feeding via as yet unidentified signals and mechanisms [68]. However, it should be noted that TfR1 is required for browning of adipocytes, thermogenesis and protection against insulin resistance, through the regulation of intracellular iron metabolism and mitochondrial function [69].

Within cells, ferritin is involved in iron storage; therefore, increased ferritin synthesis has a potential protective function by limiting the production of free radicals in redox biology and inducing the expression of anti-inflammatory cytokines in immune responses [70]. By contrast, experimental work in mice and cellular models suggests that ferritin might also act as pro-inflammatory molecule (reviewed in [70]). Consistent with this finding, in a retrospective study in patients with haemochromatosis, serum levels of ferritin were a better predictor of fibrosis stage than iron content in the liver, sex, steatosis and alcohol intake, which suggests that ferritin might be involved in fibrosis rather than simply acting as a passive indicator of iron storage [71]. Whether ferritin is a bystander or a mediator of pathological processes in patients with metabolic dysfunction deserves further investigation.

A working model of the mechanism underlying altered iron metabolism that facilitates iron accumulation in the tissues of patients with MHF is presented in Figure 1.

[H2] Definition and diagnosis of MHF

R3. We propose serum levels of ferritin as the most accurate and available biomarker to non-invasively capture and grade the presence of the aforementioned iron metabolism alteration (namely MHF), associated with glucose and lipid metabolism dysregulation and with hepatic lipid accumulation (A) (CR).

[Au: Should R4 be listed here? As the following paragraph relates to this recommendation?]

There are four reasons why we suggest that the diagnosis of MHF and grading of the severity of MHF should be established according to the serum concentrations of ferritin. Firstly, the serum level of ferritin is an almost universally available and inexpensive biomarker, compared with MRI-based tissue iron quantification. Secondly, there is well-established standardization of measurements for serum levels of ferritin [72]. Thirdly, evidence shows that in the absence of acute inflammation, serum levels of ferritin reflect the severity of iron stores or iron overload [6,7,18-21]. Fourthly, serum levels of ferritin can also reflect alterations in iron metabolism related to lipotoxicity and subclinical chronic inflammation that are correlated with tissue damage independently of iron stores [14]. We named the condition MHF without referring to alterations of iron metabolism and accumulation to reflect this choice.

R4. We propose to define this condition as MHF, and to grade its severity according to serum levels of ferritin thresholds (grade 1 to 3), which will need prospective validation and optimization. When possible, serum levels of ferritin should be evaluated after at least 3 months of lifestyle changes (U) (CR).

R5. As criteria for metabolic dysfunction, we propose those matching the definition of 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 (A) (CR).

The proposed diagnostic criteria for MHF are reported in Box B. To provide a consistent and comprehensive conceptual framework, the definition of metabolic dysfunction was modelled after that used to diagnose MAFLD [73]. We propose to grade the severity of iron metabolism alterations from 1 to 3 according to the serum concentration of ferritin and, when available, hepatic concentration of iron (Box B; note that haemochromatosis, persistently increased transferrin saturation and some cancers exclude the diagnosis of MHF). We suggest re-evaluating serum levels of ferritin following at least 3 months of lifestyle counselling, including reduction of alcohol intake and optimization of pharmacological therapy, when this approach is feasible in clinical practice or research studies. This suggestion is justified by the fact that serum levels of ferritin are partially responsive to lifestyle modifications [74,75], which highlights the concept that the alterations in

iron metabolism seen in MHF might reflect lipotoxicity as well as iron stores. Lifestyle counselling should be personalized and usually includes advice on weight loss, dietary composition, cessation of alcohol intake and increasing physical activity. MHF grading needs to be validated for the ability to stratify the risk of clinical events; this validation will help to standardize outcome reports in epidemiological studies and improve stratification in therapeutic studies.

R6. Given the initial evidence that in patients with stable MHF, serum levels of ferritin might be associated with iron accumulation in tissue, we suggest the non-invasive estimation of iron concentration in the liver by MRI in clinical studies. This approach can be considered, when available, in pathophysiological studies and in clinical practice in patients with high serum levels of ferritin (grade 2, but in particular, grade 3) and/or additional clinical risk factors for iron overload. When available, iron concentration in tissues should have priority over serum levels of ferritin for grading MHF (A) (CR).

After staging of fibrosis by non-invasive approaches, as recommended by clinical practice guidelines for fatty liver disease [76], quantification of hepatic levels of iron by MRI, if available, can be used to stage MHF as a complementary approach to serum ferritin levels to better characterize iron metabolism and tissue iron stores in clinical research studies. We also suggest the non-invasive evaluation of tissue levels of iron for patients with grade 2, but especially grade 3, MHF (based on serum levels of ferritin) in clinical practice. Grade 3 MHF corresponds to DIOS, and requires non-invasive assessment of increased iron stores in the liver to enable the clinical diagnosis. In addition, precise noninvasive criteria are needed to diagnose DIOS. Non-invasive assessment of the iron content of the liver using MRI, whenever possible, is also suggested for patients with grade 2 MHF based on serum levels of ferritin. An R2* value ≥70/140 1/s would assign the patient to grade 2 MHF (that is, dysmetabolic iron accumulation) or grade 3 MHF (that is, DIOS), respectively. Iron accumulation in the liver is quantified according to expert recommendations and available clinical practice guidelines on non-invasive quantification of iron content in the liver for radiologists, as reported in Supplementary Box 1 [77-79]. Although the serum levels of ferritin and MRI signal cut-offs for grading MHF are provisional and the correlation between serum levels of ferritin and R2* is not universally validated, we propose to identify three levels of iron accumulation for a

granular stratification. Estimation of iron stores by T2/R2 relaxometry is an acceptable alternative when locally available. When MRI is not available, in patients with coexistent liver disease, quantification of liver levels of iron could be determined by direct iron measurement in histological tissue samples.

R7. We propose to define the presence of 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 (U) (CR).*

R8. Liver biopsy is not required for the diagnosis of MHF or DIOS, unless otherwise indicated for the management of associated liver disease or for specific research purposes (U) (CR).

We propose keeping the acronym DIOS for individuals with evidence of severe serum and hepatic iron load, as summarized in Table A. **[Au: The original Figure 2 has been converted to a Table; I will renumber the display items when you submit your revised text.]** Evidence from large population studies suggests that a predisposition to even a mild degree of iron accumulation can facilitate organ damage, such as cirrhosis [36,80]. Determining the relative contribution of inflammation or iron overload remains a major challenge in assessing patients with MHF. We believe that liver biopsy is not required for the diagnosis of DIOS, unless otherwise indicated for the management of associated liver disease or for specific research purposes.

The most frequent differential diagnoses to be considered in clinical practice are listed in Supplementary Table 2. The suggested diagnostic and staging work-up for patients with suspected MHF is reported in Supplementary Table 3.

[H2] Clinical management

R9. The clinical management should be focused on lifestyle factors associated with increased risk of cardiometabolic risk factors (such as caloric intake and dietary patterns, alcohol intake, fructose and salt intake and sedentary lifestyle) and the pharmacological control of cardiovascular risk factors (U) (CR).

The association between serum levels of ferritin and the risk of cardiometabolic diseases is reported in Supplementary Table 4. Increased serum concentrations of ferritin and hepatic iron stores have been associated with the risk of type 2 diabetes mellitus, cardiovascular damage, and several phenotypes related to liver disease (inflammation, fibrosis and hepatocellular carcinoma; evidence detailed and referenced in Supplementary Table 4). This finding highlights the unmet need to assess for and treat cardiovascular risk factors in patients with MHF. The promotion of a healthy lifestyle, a balanced diet low in processed foods, regular exercise and limited alcohol consumption are the cornerstone of the clinical management of this condition.

R10. In patients with MHF and DIOS, iron depletion therapy should be considered as an experimental therapy to be tested in well-powered controlled trials (U) (CR).

Several studies have suggested that increased stores of iron in the body might have a causal role in determining organ damage, and that iron stores could be a modifiable risk factor in patients with metabolic disorders. The main results of controlled studies testing the effect of iron depletion in patients with hyperferritinaemia associated with metabolic dysfunction are shown in Supplementary Table 5. Case–control studies suggested that phlebotomy might decrease insulin resistance in patients with fatty liver and metabolic dysfunction [81,82]. Case–control studies also highlighted an improvement in liver damage (detected by liver enzymes and histology) in people with hyperferritinaemia who underwent and maintained iron depletion [83,84]. Furthermore, iron depletion was associated with a reduced risk of cancer in patients with peripheral arterial vascular disease, mostly smokers with severe atherosclerosis [85]. However, in a randomized trial of patients with DIOS, iron depletion did not improve insulin resistance in the short-term but was associated with reduced levels of liver enzymes (that is, alanine aminotransferase and aspartate transaminase) [75]. A meta-analysis, which was limited by the low number and heterogeneity of the studies considered (in terms of selection criteria, baseline iron stores, outcomes and duration of observation), concluded that in patients with non-alcoholic fatty liver disease (NAFLD) with or without DIOS, iron depletion only had a minor effect on the improvement of alanine aminotransferase levels, but did not affect insulin resistance and liver histology compared with lifestyle changes alone [86]. Therefore, outside

clinical studies, iron depletion can only be considered in patients with confirmed severe hepatic iron accumulation (grade 3) associated with steatohepatitis or clinically significant liver fibrosis unresponsive to therapy.

The recommended clinical management for MHF based on the currently available evidence is reported in Figure 2.

[H2] The role of blood donation

R11. 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 (U) (CR).

Approximately 250 mg of iron are removed with each 450 ml blood donation, which accounts for about 30% of the average iron stores in the liver in men and nearly 80% in women. On regular blood donation, individuals usually reach stability of iron balance at a lower level of body iron stores [87]. On this basis, since the proposal of the iron-heart hypothesis on cardiovascular disease in the early 1980s [88], a number of studies have been conducted to evaluate if regular blood donation can counteract iron toxicity and cardiometabolic complications by preventing iron overload and cardiometabolic complications. In the general population, frequent blood donors (defined as 2–10 donations per year) had increased insulin sensitivity, decreased insulin secretion and significantly lower serum levels of ferritin than nondonors, with both groups having similar blood concentrations of haematocrit and haemoglobin [89].

A large cohort study from the USA has reported a protective effect (OR 0.67) of blood donation in terms of cardiovascular morbidity in non-smoking men[90]. The benefit of donation was greater in those with higher serum levels of LDL cholesterol than in those with lower levels, while no significant effect of blood donation was seen in women. This finding is in agreement with the iron-cardiovascular disease hypothesis, which postulates that the protective effect of phlebotomy would be more prominent in men, who have higher iron load than women [88]. Furthermore, an 88% reduced risk of myocardial infarction was also observed in a prospective epidemiologic study from eastern Finland, which included 2,862 men aged 42–60 years (153 donors and 2,529 non-donors), who were followed up for an average of 9

years [91]. However, the results of these studies could have been biased by the selection of fairly healthy people for blood donation, the so-called healthy donor effect [92]. Subsequent studies adjusted for the healthy donor effect gave conflicting results, which further underscores the difficulties of drawing conclusions from observational and cohort studies on this topic [93-97]. Unfortunately, a controlled randomized clinical trial on the effects of blood donation on cardiometabolic morbidity and mortality seems to be almost impossible to conduct, and the question will have to be addressed by alternative study designs.

[H1] Future perspectives

R12. Additional studies are required to define the specific genetic and environmental risk factors for MHF and DIOS development (U).

R13. Additional studies are required to define the correlation between serum levels of ferritin and hepatic iron content determined by MRI in patients with MHF (U).

R14. 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 (U).

The main future challenges in the field are reported in Supplementary Table 6. We listed specific items related to the definition, diagnosis, epidemiology, genetics, pathophysiology and treatment, including suggestions on the optimal study design that might be fostered by the implementation and prospective validation of a common definition of MHF. The main goals from a pathophysiological point of view will be to clarify what determines serum levels of ferritin and iron accumulation in individuals with metabolic dysfunction, and clarifying the relationships among alterations in iron metabolism, lipotoxicity, inflammation, insulin resistance and organ damage. From a clinical perspective, the goal should be to determine whether assessment of serum levels of ferritin and iron stores in the body has clinically meaningful additive prognostic value and whether reducing

iron stores protects against organ-specific complications and improves clinical outcomes in individuals with metabolic dysfunction.

R15. Clinical studies evaluating iron depletion in patients with MHF or DIOS should consider, as main outcomes, biomarkers that are closely linked to clinical events and take into account the perceived quality of life. These outcomes should be assessed after an adequate duration of follow-up, at least 3 months after achievement of iron depletion in the active arm (A).

Further multicentre randomized studies with simpler inclusion criteria and longer follow-up than those currently available, and eventually evaluation of hard clinical outcomes (that are more closely associated with major clinical events and/or mortality than liver enzymes or insulin levels), are necessary to draw more reliable conclusions. In particular, we make several proposals (as reported in Supplementary Table 4). MHF should be stratified by severity in randomized controlled studies. Serum concentrations of ferritin after at least 3 months of lifestyle change should be among the inclusion criteria of these studies. Iron depletion protocols and methods for iron maintenance should be standardised, and the possible use of supportive therapies (e.g. folate supplementation) should be clarified. Outcomes should be examined at least at 3 months after achievement of iron store normalization. Observational studies in blood donors that control for donation frequency and propensity score should be considered. The incidence of type 2 diabetes mellitus, major cardiovascular events, cancer and liver events (such as progression of liver fibrosis) should be considered as outcomes. Sustained modification of diet and lifestyle habits remain the first therapeutic interventions in patients with MHF, together with control of cardiovascular risk factors by pharmacological approaches when necessary [17].

[H1] CONCLUSIONS

Although not yet universally recognized as a distinct clinical entity, MHF and its disease stages might identify a subset of individuals in whom alterations of carbohydrate and lipid metabolism extend into a disrupted iron homeostasis, possibly triggered by insulin resistance, lipotoxicity and subclinical chronic systemic inflammation. Compared with patients with metabolic dysfunction without MHF, these individuals seem to be at higher risk of type 2 diabetes mellitus and of hepatic, cardiovascular and neoplastic diseases, independently of

classic risk factors. Conflicting data suggest the possibility that iron accumulation in tissues is causally implicated in the pathogenesis of these cardiometabolic diseases. Although this hypothesis requires evaluation in robust clinical studies, the potential to reduce the complications of metabolic disorders by achieving and maintaining a state of near iron depletion deserves further investigation. As iron deficiency is associated with fatigue and impaired quality of life, and with worse outcomes in patients with heart failure [98] and type 2 diabetes mellitus [99], these studies must include an assessment of patient-related outcome measures, and symptoms of heart failure should be assessed at baseline. In this context, non-invasive multi-organ MRI evaluation could enable the simultaneous assessment of lipid levels in the liver, inflammation, fibrosis and hepatic and splenic iron concentration, as well as cardiac function [98].

However, there are two major obstacles hindering the design and implementation of randomized controlled clinical trials in this field. The first obstacle is the difficulty in finding sufficient funding for conventional iron depletion by phlebotomy, and the second one is the impossibility to run blind studies together with the presence of many confounders by using phlebotomy. There are two strategies to overcome the difficulties in conducting randomized clinical trials. The first one is to invest more in basic and translational research in the field, aiming at better defining the molecular pathways underlying MHF and pharmacological approaches to improve organ damage by modulating iron metabolism. The second one is to set up a robust research framework and generate new data to create a strong rationale for supporting these studies by public funding. In this regard, an apparent weakness (that is, the current necessity to rely on phlebotomy to achieve normalization of iron stores) might turn into a compelling argument for public health systems to support research evaluating this approach. Indeed, there is currently a global shortage of blood donations and blood products that has been worsened by the COVID-19 pandemic. Expanding the pool of blood donors, while assessing whether regular donation might protect against the development of cardiovascular, liver and neoplastic disorders, might also help overcome this threat to public health [100]. We believe that the current Consensus statement enables an important first step in this direction.

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Author contributions

L.V., E.C. and D.P. contributed to all aspects of the manuscript. L.A.A., E.A., M.A., E.B.-J., E.B., J.M.F.-R., D.G., H.H., K.K., G.L., F.L., K.M., A.Pi., C.W.S., P.S, E.A.T. and H.Z. provided substantial contributions to the discussion of the content and reviewed and/or edited the manuscript before submission. S.A., B.H., D.M., M.U.M., P.P., J.D.R., L.S., A.Pa. and F.V. provided substantial contributions to the discussion of the content, wrote the manuscript and reviewed and/or edited the manuscript before submission. M.-H.Z. researched data for the article, provided substantial contributions to the discussion of the content and reviewed and/or edited the manuscript before submission.

Competing interests **[Au: Some of the authors have first or middle initials in the author list; I have added them in, but please check they are all correct.]**

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Supplementary information

Supplementary information is available for this paper at https://doi.org/10.1038/s415XX-XXX-XXXX-X

TABLES

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Box A. Contributing factors [Au: Titles need to be short, so I have added a new one and moved the original title to a paragraph below.]

These factors contribute to or are associated with increased levels of ferritin or the development of metabolic hyperferritinaemia (MHF) and subsequent progression to iron accumulation (DIOS) in individuals with metabolic dysfunction or fatty liver disease.

Genetic

- Male sex [4,7,30]
- Heterozygous presence of the *HFE* p.C282Y pathogenic variant or homozygous presence of the p.H63D variant or, in particular, compound heterozygosity for p.C282Y/p.H63D variants[7,30,101]
- *PCSK7* variants [102]
- Absence of *TMPRSS6* p.A736V variant [37,103]
- Heterozygous presence of *SERPINA1* PiZ and PiS pathogenic variants [104]
- Heterozygous pathogenic variants of β-globin gene (*HBB*), that is beta-thalassemia trait [18]
- Rare *NMBR* variants [105]
- Heterozygous pathogenic variants of the gene encoding ceruloplasmin [31]

Acquired

- Severity of insulin resistance [3-11,106]
- Severity of fatty liver disease [6,7,18-21]
- Ageing [4,7,30]
- Altered regulation of iron metabolism (increased absorption and cellular retention) associated with lipid accumulation [14,40-42,107]
- Iron accumulation in the liver[7,9,20,21]
- Moderate alcohol intake; more than 30 g per day in men and 20 g per day in women[7]
- Hepatic copper deficiency and reduced ceruloplasmin activity [51,52]

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[Au: At nearly 900 words, this is far too large for a Box or Table. The limit for Text Boxes is 300 words. I suggest splitting it into 2 or 3 separate boxes, and reducing the size of the footnotes. Please let me know how you would like to split the text, and please make cuts to the footnotes.]

Box B. Proposed updated diagnostic criteria for metabolic hyperferritinaemia.

- Serum concentrations of ferritin: circulating levels of ferritin >300 ng/ml in men and >200 ng/ml women[a]
- Plus evidence of fatty liver: by liver biopsy, imaging (including MRI, ultrasonography, computed tomography), continuous attenuation parameter by transient elastography or non-invasive biomarkers or scores (such as fatty liver index, limited to epidemiological studies [108,109]).
- Or type 2 diabetes mellitus and/or obesity (adjusted for ethnicity, BMI>30 kg/m^2 in people of European origin).
- Or ≥2 features of altered metabolism associated with insulin resistance [16]
    - Overweight (adjusted for ethnicity, BMI >25 kg/m^2 in people of European origin) or increased abdominal circumference (sex and ethnicity adjusted, >102 cm in men and >88 cm women of European origin).
    - Increased circulating levels of triglycerides (>150 mg/dl).
    - Low HDL cholesterol (<45 mg/dl in men and <55 mg/dl in women).
    - Increased fasting levels of glucose (>100 mg/dl).
    - Arterial hypertension (>130/85 mmHg or use of anti-hypertensive agents).
    - Evidence of fasting hyperinsulinaemia or insulin resistance (locally validated, such as with a HOMA-IR index >2.7).

Exclusion criteria

- Haemochromatosis, genetically confirmed, or evidence of increased body stores of iron with persistently increased transferrin saturation (>50%) or other genetic disorders affecting iron metabolism: these include iron metabolism disorders (such as ferroportin disease), anaemias with iron loading (such as thalassaemias and dyserythropoietic anaemias) and haemolytic disorders.
- High alcohol intake (>60 g per day in men and >40 g per day in women within the past 6 months) [b].
- Anaemia, when not mild and related to thalassaemia trait and/or having other haemoglobin variants (such as sickle haemoglobin, HbS)[c].
- Red blood cell transfusion therapy or previous sustained red blood cell transfusions (such as after major trauma or critical illness) or recent (within 5 years) iron infusion therapy.
- End stage renal disease or dialysis.
- Other forms of secondary iron overload, for example due to exposure to welding fumes [110].

Re-evaluate after resolution of trigger[d]

- Inflammatory disorders (demonstrated active infections, active autoinflammatory disorders, C-reactive protein levels higher than the upper limit of normal range, advanced neoplasia, sepsis and multiorgan failure)[e].
- Acute liver injury (liver enzymes >5 times the upper limit of normal).
- Moderate alcohol intake (<60 g per day in men and <40 g per day in women) or binge drinking in the preceding 6 weeks[f].

- Poorly controlled diabetes mellitus.
- Oral iron supplementation, multivitamins with iron, vitamin C supplements.

With or without iron accumulation

- In the absence of non-invasive evaluation, iron accumulation might be suspected if ferritin is >550 ng/ml after lifestyle changes, which is identified as the best threshold to discriminate the presence of hepatic iron accumulation in patients with NAFLD [31,82].
- In clinical studies and in clinical practice in patients with ferritin >550 ng/ml, iron accumulation can be determined on evidence of histological iron staining at liver biopsy (non-parenchymal or mixed pattern) or hepatic iron concentration >2,000 μg/g dry liver tissue, or iron removed to reach depletion >3.5 g in men or >2.5 g in women, or increased hepatic iron stores, as non-invasively detected by MRI (according to local clinical protocols) with R2*>70 1/s, as the preferred fist-line approach [19].

Metabolic hyperferritinaemia grading[d]

- Grade 1: no significant iron accumulation (ferritin <550 ng/ml, R2*<70 1/s equivalent to <36umol/g of hepatic iron)
- Grade 2 (dysmetabolic iron accumulation): mild iron accumulation (ferritin levels 550–1000 ng/ml, or metabolic hyperferritinaemia and R2* 70-140 1/s, equivalent to 36–74 μmol/g of iron in the liver)
- Grade 3 (dysmetabolic iron overload syndrome[g]): moderate or severe iron accumulation (ferritin >1,000 ng/ml, or metabolic hyperferritinaemia and R2*>140 1/s, equivalent to >74 μmol/g of iron in the liver).

[a] This threshold needs to be validated in each population. In fact, this should be itself a target for research. [b] In line with the definition of fatty liver associated with metabolic dysfunction, low or even moderate at-risk alcohol intake (>30 g per day in men or >20 g per day in women) is considered a cofactor, but it does not rule out the contribution of metabolic dysfunction to hyperferritinaemia and the possibility to diagnose dysmetabolic iron accumulation. [c] Presence of mutations in heterozygosity affecting iron or erythropoiesis are not considered as an exclusion criterium. [d] Grading should be preferentially determined after at least 3 months of lifestyle change counselling, when it is feasible (it is not mandatory for cross-sectional epidemiological studies or in clinical centres when this is not logistically feasible). When available, quantification of iron accumulation in the liver by MRI should have the priority over the evaluation of serum levels of ferritin to grade metabolic hyperferritinaemia. [e] C-reactive protein persistently higher than the upper limit of normal range is considered an exclusion criterion and should prompt the evaluation of alternative diagnoses (see Supplementary Table 2). [f] The hypothesis to be tested is that alcohol intake below this threshold can be considered as a cofactor rather than an exclusive cause of hyperferritinaemia, in line with the MAFLD definition. [g] Diagnosis of dysmetabolic iron accumulation and dysmetabolic iron overload syndrome requires non-invasive demonstration of hepatic iron accumulation. MRI: magnetic resonance imaging; HOMA-IR: homeostasis metabolic assessment insulin resistance index.

[Au: Our articles can have a maximum of 7 display items, and the article currently has 8. Given that the previous Box is going to add 1 or 2 more display items, we need to remove 2-3 display items. I suggest moving Table 3 to the Supplementary information. Please add this (and the references) to your SI document.]

[Au: As noted above, we need to reduce the number of display items, so I suggest moving Table 4 to the Supplementary information. Please add this (and the references) to your SI document.]

FIGURE LEGENDS

Figure 1. Pathophysiology of metabolic hyperferritinaemia and associated iron accumulation. Hepatic lipid accumulation (steatosis) and alterations of lipid metabolism in the liver are conditions associated with systemic insulin resistance, and lead to lipotoxicity and local inflammation, inducing the synthesis of ferritin, a molecule with anti-oxidant activity. Within this context, excess levels of lipids and iron in the diet lead to increased circulating levels of iron (Fe), especially in male individuals with a permissive genetic background. Due to the presence of subclinical inflammation downregulating ferroportin 1, iron is accumulated not only in hepatocytes, but also in Kupffer cells and hepatic stellate cells, triggering hepatocellular damage, ferroptosis, inflammation and fibrogenesis. This process triggers progressive liver disease, but spillover of iron from the liver might also worsen insulin resistance in adipose tissue and impair the secretion of appetite-controlling peptides, thereby facilitating the progression to type 2 diabetes mellitus and its complications. The figure represents the main pathophysiological pathways leading to metabolic hyperferritaemia in patients with metabolic dysfunction (not all the extrahepatic and intrahepatic interactions between lipid, glucose and iron metabolism are depicted).

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Table A. Figure 2. The spectrum of iron metabolism in individuals with metabolic dysfunction.

Factor	Normal iron metabolism	MHF		
		Stage 1 (MHF)	Stage 2 (dysmetabolic iron accumulation)	Stage 3 (dysmetabolic iron overload syndrome)
Ferritin (ng/ml)	50 to upper limit of normal	Upper limit of normal to 550	550–1,000	>1,000
Hepatic iron stores (R2* 1/s)	<70	<70	70–140	>140
Iron stores (versus normal)	Normal	Normal	Increased	Very increased
Organ damage related to iron	Absent	Usually absent	Rare	Present

Metabolic hyperferritinaemia (MHF) is defined in the presence of metabolic dysfunction when ferritin levels are above the upper limit of normal (>300 ng/ml in men and >200 ng/ml in women). MHF can next be staged to estimate iron accumulation and tissue damage, according to ferritin levels. Grading should be preferentially determined after at least 3 months of lifestyle change counselling, when it is feasible (it is not mandatory for cross-sectional epidemiological studies or in clinical centres when this is not logistically feasible). When available, quantification of hepatic iron accumulation by MRI should take priority over the evaluation of serum levels of ferritin to grade MHF.

Figure 2. Clinical management of metabolic hyperferritinaemia. The diagnosis, based on the evaluation of inclusion and exclusion criteria, is followed by staging of iron accumulation (by ferritin and if available by MRI) and of organ damage. In addition to the current therapy for metabolic dysfunction, iron depletion therapy can be considered for patients with the most severe iron stores or within clinical trials. Iron stores are then monitored non-invasively.

Table of contents

This Consensus Statement discusses the relationship between hyperferritinaemia and iron accumulation in individuals with metabolic dysfunction. The authors propose an updated definition and a provisional staging system for metabolic hyperferritinaemia, highlight research gaps and provide suggestions the design and outcome measures for future studies.