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REVIEW

New drugs

Iron deficiency and heart failure: diagnostic dilemmas and therapeutic perspectives

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Iron is a micronutrient essential for cellular energy and metabolism, necessary for maintaining body homoeostasis. Iron deficiency is an important co-morbidity in patients with heart failure (HF). A major factor in the pathogenesis of anaemia, it is also a separate condition with serious clinical consequences (e.g. impaired exercise capacity) and poor prognosis in HF patients. Experimental evidence suggests that iron therapy in iron-deficient animals may activate molecular pathways that can be cardio-protective. Clinical studies have demonstrated favourable effects of i.v. iron on the functional status, quality of life, and exercise capacity in HF patients. It is hypothesized that i.v. iron supplementation may become a novel therapy in HF patients with iron deficiency.

Keywords Heart failure • Iron deficiency • Soluble transferrin receptor • Hepcidin • Prognosis • Exercise capacity

Introduction

Iron deficiency (ID) is the commonest nutritional deficiency worldwide, affecting more than one-third of the population.[1–4] Although ID is traditionally linked to anaemia,[2–4] ID is more prevalent and its economic consequences relevant, although not commonly acknowledged,[1,2,5,6] ID adversely affects the function and limits the survival of living organisms at every complexity level[1,3,6] (*Figure 1*).

Iron deficiency is a complication of chronic diseases (e.g. inflammatory bowel disease, Parkinson's disease, rheumatoid disease, chronic renal failure), irrespective of concomitant anaemia.[1,7–11] The first reports on ID in cardiovascular disease were published >50 years ago.[12,13] Iron deficiency coincided with sympathetic activation,[14] left ventricular hypertrophy,[14–16] dilatation,[16,17] compromised haemodynamics and symptomatic heart failure (HF).[12,13] These findings have been mainly forgotten over the years.

In the last decade, anaemia was recognized as an important co-morbidity in HF, a factor limiting physical activity, responsible for a poor quality of life, and a predictor of unfavourable outcomes.[18–22] Iron deficiency generated interest as a cause of anaemia.[23–25] Iron deficiency was hypothesized to be the cause of erythropoietin resistance in HF,[26–28] which could be responsible for the unsatisfactory effects of erythropoietin therapy in HF.[29–31]

Physiological role of iron

Iron is a metabolically active micronutrient with unique biochemical features.[1,3,32–35] Iron changes between two oxidative states, bivalent ferrous (Fe^{2+}) and trivalent ferric (Fe^{3+}) iron.[1,32–36] Hence, it can be a cofactor for enzymes and the catalyst of biochemical reactions, an element of proteins with distinct cellular functions (as enzymes, and transport and structural proteins).[1,32–36]

Iron plays a crucial role in oxygen transport (haemoglobin component), oxygen storage (myoglobin component), cardiac and skeletal muscle metabolism (component of oxidative enzymes and respiratory chain proteins), synthesis, and degradation of proteins, lipids, ribonucleic acids (enzyme component),[1,3,32–34,37,38] and mitochondrial function.[38–40]

Iron is required for optimal haematopoiesis.[3,28,33,41] The majority portion of it is taken up by erythroblasts and reticulocytes for

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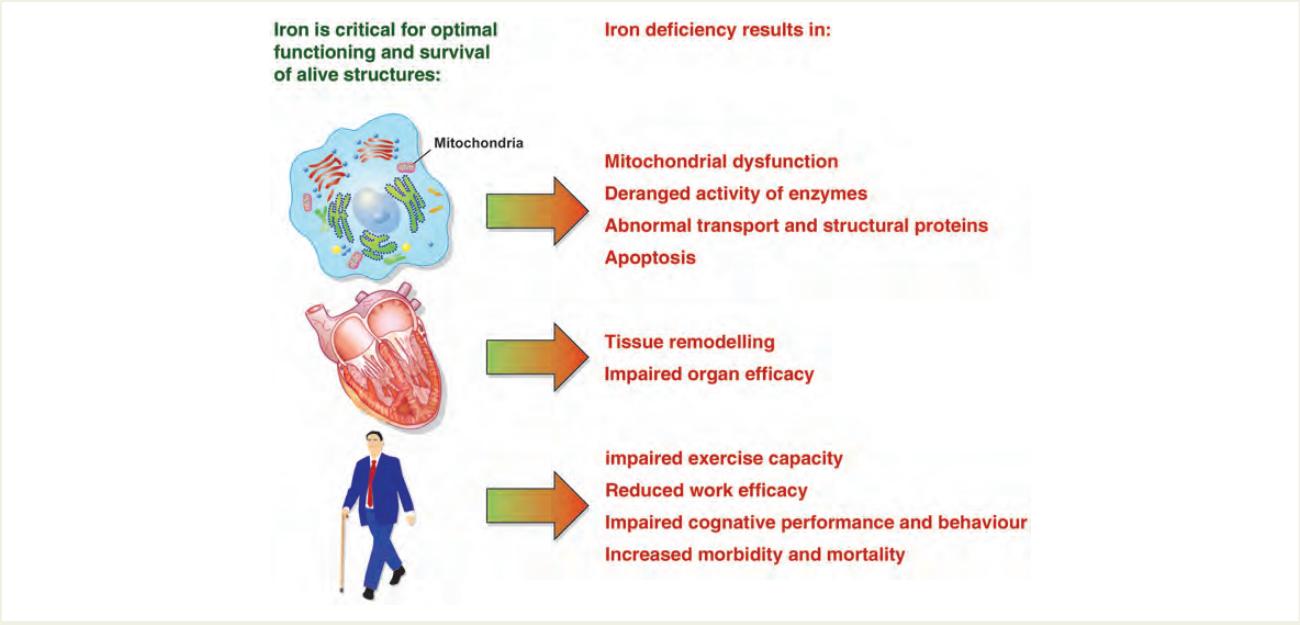

Figure 1 Importance of iron for functioning and survival across all levels of complexity of living structures.

haemoglobin synthesis.[3,28,33,41] Iron deficiency results in resistance to haematopoietic growth factors (e.g. erythropoietin), and impairs the differentiation and maturation of all types of haematopoietic cells.[26,27,33,41,42]

In spite of its unquestionable role for optimal haematopoiesis, iron is indispensable for the maintenance of cellular energy and metabolism of extra-haematopoietic tissues.[1,3,32–34,37,38] Cells with a high mitogenic potential (neoplastic, haematopoietic, immune) and high-energy demand (hepatocytes, adipocytes, skeletal and cardiac myocytes, renal cells) are particularly sensitive to depleted iron supplies and/or abnormal iron utilization.[1,3,32,35,37] This is important in HF, as abnormal energy generation and utilization in the myocardium and the peripheral tissues (e.g. skeletal muscles) contribute to HF pathophysiology.[43–47]

Iron excess accumulates in cells, and at higher concentrations generates oxidative stress[48–53] and triggers cardiomyocyte necrosis,[54] whereas at lower concentrations stimulates inducible nitric oxide synthase activity and through increased NO production induces signalling pathways promoting cell survival.[54]

Major pathways of iron turnover

Average iron intake is 10–20 mg/day, but only 10–20% of dietary iron is normally absorbed using specific transport systems, mainly by duodenal enterocytes.[55–58] There is no pathway for iron excretion. Under normal conditions, the same iron amount is lost from skin desquamation, sloughing of epithelial cells, and bleeding.[55–58]

Dietary iron in two forms, inorganic (non-haem) and organic (haem), is absorbed using distinct transmembrane transport systems consisting of three elements: a specific transport protein complex, an enzyme changing the oxidative iron state, and regulatory proteins.[55–58] In the body, intracellular iron exists in the ferrous form (Fe^{2+}) and extracellular circulating iron in the ferric form (Fe^{3+}).[55–58]

Inorganic dietary iron is absorbed by the apical surface of duodenal enterocytes via the divalent metal transporter 1 (DMT1) and accompanying membrane ferrireductases reduce ferric to ferrous iron.[55–59] Haem iron is absorbed through a haem carrier protein, and an inducible haemoxigenase 1 reduces iron before entering the cytosol.[55–58] Iron is transported from the cytosol to the circulation by the basolateral surface of enterocytes using ferroportin and an accompanying membrane hephaestin oxidizes ferrous into ferric iron, which is released into the circulation and bound to transferrin.[55–59]

There are two major pools of iron, utilized and stored (*Figure 2*). Utilized iron consists of circulating and intracellular iron.[55–58] Circulating ferric iron is bound to transferrin, which serves as a reservoir of soluble iron, delivers iron to target cells, and neutralizes the free-radical-generating properties of iron.[55–58] Iron bound to transferrin enters the target cells using transferrin receptor type 1 (TfR 1)-mediated endocytosis, the major pathway of iron import.[55–59] The vast majority of intracellular iron is in erythrocyte haemoglobin and circulating reticulocytes.[55–58] Other cells contribute to specific functions in iron turnover, e.g. enterocytes for dietary absorption, macrophages eliminate senescent erythrocytes, hepatocytes release proteins regulating iron metabolism (hepcidin).[55–58]

Stored iron is in liver, bone marrow, and spleen cells in a non-toxic form in ferritin shells, which is secreted to the extracellular compartment.[55–61] In iron overload or inflammation, the tissue expression of ferritin increases.[55–61] However, the precise functions of intracellular and extracellular ferritin and the source of circulating ferritin remain unclear.

Iron pools interact with each other, and iron can be transferred between these compartments using tightly regulated mechanisms.[55–60]

Within iron homoeostasis, one can distinguish conceptually two dimensions of iron traffic, i.e. one related with iron absorption and its transport between tissues in the whole organism (systemic iron

metabolism), and the other related to iron transport between organelles within the cell (intracellular iron metabolism).[57,60] Each has distinct regulatory mechanisms. Systemic iron metabolism is controlled by mechanisms involving hepcidin and its receptor (ferroportin), whereas intracellular iron metabolism is orchestrated by a complex of iron-regulatory proteins.[57,60] Hepcidin, a small peptide hormone synthesized mainly by hepatocytes, is considered the major regulator of iron metabolism and a part of an innate immune response.[62–67] Circulating hepcidin interacts with its specific transmembrane receptor (ferroportin) on target cells, which causes: (i) reduced expression of proteins involved in transmembrane iron import to enterocytes, (ii) internalization of ferroportin, the only protein able to export intracellular iron.[57,62–67] Hence, hepcidin blocks intestinal absorption of iron, and diverts iron from the circulation into the reticuloendothelial system.[66,67] Decreased intestinal iron absorption together with its accumulation in the reticuloendothelial stores reduces the availability of iron to target tissues.[57,62–65] Hepcidin synthesis by hepatocytes is precisely regulated in order to optimize and synchronize iron metabolism, and to react to changing tissue demands for iron. Major stimuli decreasing hepcidin expression in the liver and its release into the circulation are: depleted iron stores, hypoxia, and ineffective erythropoiesis, whereas inflammation produces the opposite effect.[57,60,62–65,68]

Diagnosis and classification of iron deficiency

Two types of ID need to be distinguished: absolute, and functional ID[3,57,69–71] (*Figure 3*).

Absolute ID reflects depleted iron stores, often with intact iron homoeostasis mechanisms and erythropoiesis.[69,70] The commonest causes are: low-dietary iron, impaired gastrointestinal (GI) absorption and GI blood loss, menorrhagia (*Figure 3*). Functional ID reflects inadequate iron supply to meet the demand despite normal or abundant body iron stores, because iron is trapped inside cells of the reticuloendothelial system and is unavailable for cellular metabolism[69,70] (*Figure 3*). It is believed to be mainly caused by pro-inflammatory activation with hepcidin overproduction (see above).[62,64–67]

Approximately 80% of the total body iron is in the erythron, being a component of haemoglobin.[28,33,41] Reduced iron delivery to erythroblasts and reticulocytes limits erythropoiesis, and ID is the commonest cause of anaemia.[3,28,41] Diagnostic algorithms have been developed to optimize the detection and classification of ID, and to monitor iron stores to provide adequate and optimal management of anaemia.[69–75]

The *gold standard* for evaluating iron stores in target tissues is a bone marrow biopsy.[70,72–76] Recently, Phiri *et al.*[77] proposed a histological grading by iron smear assessment with separate detection of iron in macrophages (stored iron) and erythroblasts (utilized iron), differentiating between a normal status, absolute ID, functional ID, and combined functional and absolute ID. The invasiveness of bone marrow biopsy limits its use and can be replaced by the measurement of several blood biomarkers to show iron status indirectly in most clinical scenarios[69,70,72,73,78] (*Figure 4*).

Absolute ID reflects depleted iron stores, hence its diagnosis is based on the measurement of circulating ferritin, a reliable surrogate of stored iron quantity, which originates from iron-storing cells (mainly hepatocytes and reticuloendothelial cells)[69,70,72,73,78] (*Figure 3*). There is a linear relationship between serum ferritin and ferritin expression in iron storage tissues. Currently, the generally accepted serum ferritin cut-off level to diagnose absolute ID is <30 μg/L,[70,72,73] although stricter cut-off values were used previously (12–15 μg/L).[79] Both intracellular iron accumulation and inflammation stimulate the tissue expression of ferritin and increase its blood level. In such cases, for the diagnosis of absolute ID, a higher serum ferritin cut-off value is used (e.g. 100 μg/L).[69,72]

Circulating iron bound to transferrin (TIBC, total iron binding capacity—by transferrin) reflects the amount of iron available for metabolizing target cells.[69,70,72,78] Importantly, neither serum iron nor serum transferrin alone should be used as biomarkers of iron status. Instead, transferrin saturation (Tsat), the per cent of transferrin that has iron bound to it (ratio of serum iron and TIBC $\times$ 100), is recommended. Reduced Tsat ($<20\%$) is considered a surrogate of insufficient iron available for metabolizing cells.[69,70,72,78] With malnutrition accompanying chronic diseases, liver synthesis and blood transferrin levels may be low, which can artificially increase Tsat disproportionate to the iron content.[69]

When serum ferritin is between 100 and 300 μg/L (which is frequent in patients with chronic diseases with pro-inflammatory activation), the diagnosis of ID is more complex. Such values are usually associated with normal/slightly increased intracellular iron stores and the diagnosis of absolute ID cannot be made.[69,70,72,78] If there is restricted iron delivery to target cells (reduced Tsat $<$ 20%), functional ID can be diagnosed.[69]

Therefore, in chronic diseases, absolute ID is typically diagnosed with higher cut-off ferritin values (i.e. <100 μg/L) and distinguished from functional ID, diagnosed with normal serum ferritin (100–300 μg/L) and low Tsat ($<20\%$).[69] Such a definition of ID has been applied in HF syndrome, including clinical trials.[80,81]

Iron plays a critical role in erythropoiesis, being incorporated into erythroblasts and reticulocytes.[28,33,41] Restricted delivery to the erythron can be detected in peripheral blood using indices of so-called iron-restricted erythropoiesis.[33,69,70,73,78,82] Reticulocytes are the earliest erythrocytes released into circulating blood and are present for only 1–2 days. Reduced reticulocyte haemoglobin content (<28 pg) is an early indicator of iron-restricted erythropoiesis.[69,70,78,82] Reticulocyte haemoglobin content is also an early indicator of the response to iron therapy, increasing within 2–4 days after i.v. iron therapy. Later indicators of iron-restricted erythropoiesis are: increased percentage ($>2.5\%$) of hypochromic erythrocytes [red blood cells (RBCs)] and an increased RBC zinc protoporphyrin, a product of abnormal haem synthesis.[69,70,78] Among the last parameters to change with iron-deficient erythropoiesis are the basic haematological indices: haemoglobin level, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration with the picture of microcytic hypochromic anaemia.[28,33,41,70,73–75,78]

The red cell distribution width (RDW) reflecting MCV heterogeneity (quantitative index of anisocytosis, i.e. the percentage

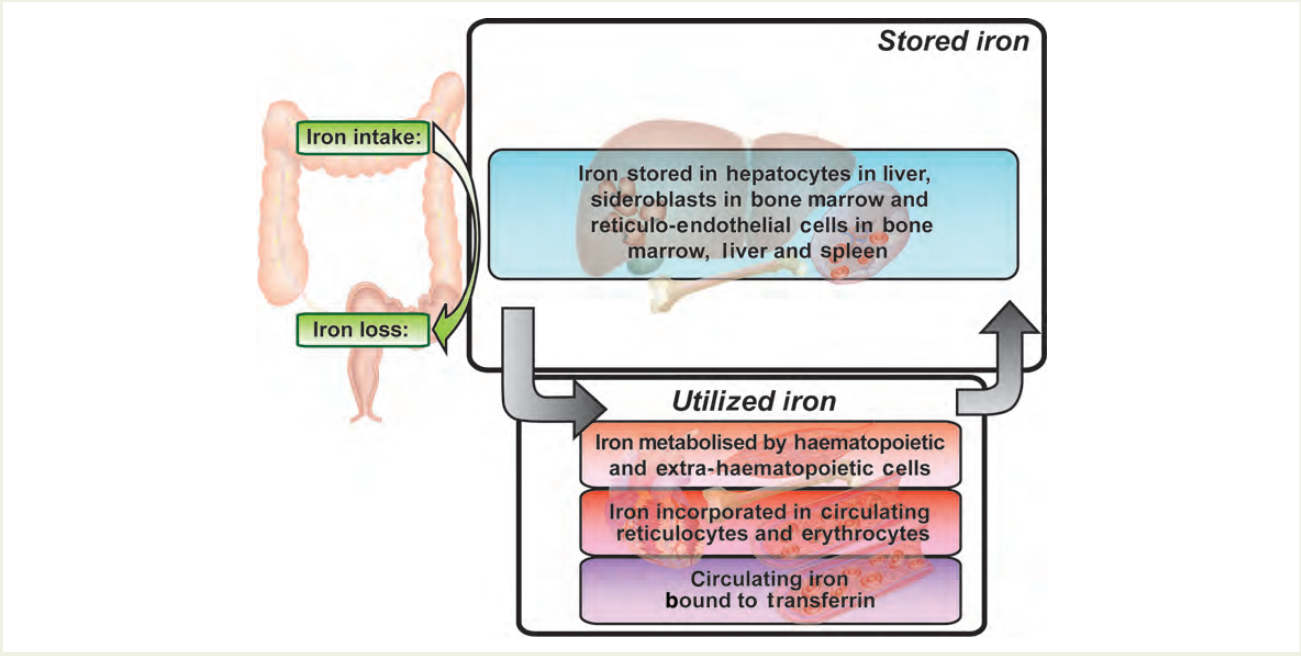

Figure 2 Major pools of utilized and stored iron in the body.

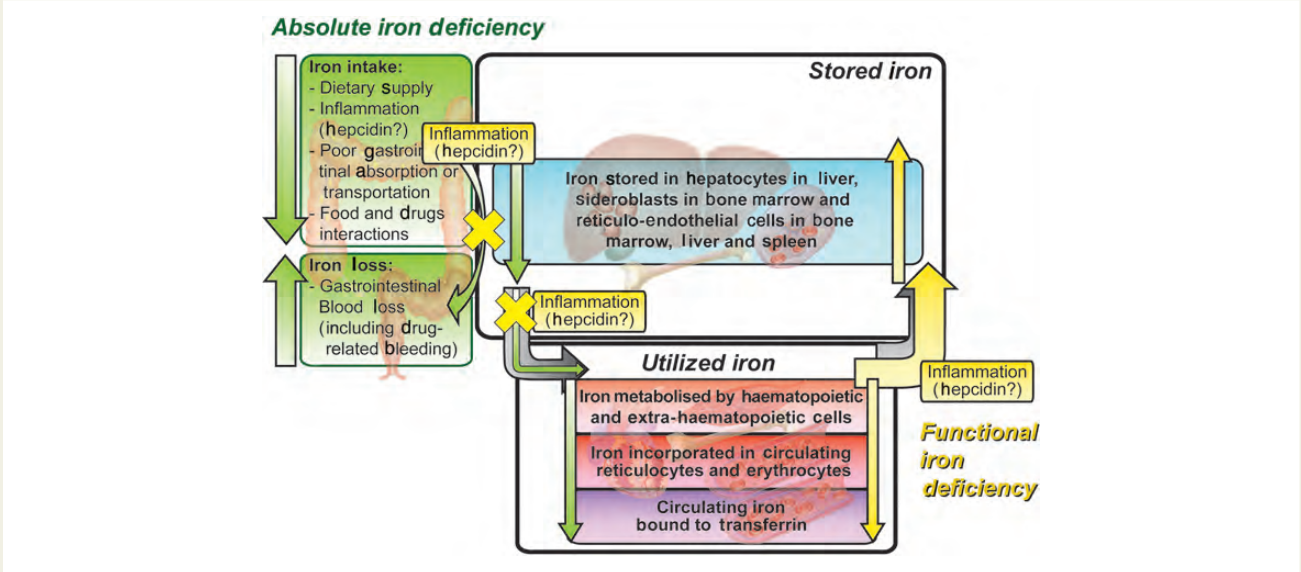

Figure 3 The concept of absolute and functional iron deficiency.

coefficient of MCV variation) can be considered another parameter of ID.[78,83,84] Increased RDW is, however, typical not only for anaemia due to ID, but also anaemia resulting from deficiencies in vitamin B_{12} and folic acid, of chronic diseases and sideroblastic anaemia.[77,83,84] In HF patients, there are associations between high RDW, and reduced haemoglobin, low MCV, reduced Tsat,[85] increased mortality and hospitalization rates.[85–87]

Owing to pathophysiological links and overlaps in regulatory mechanisms of erythropoietin and iron metabolism,[26–28] subjects with ID frequently have increased circulating erythropoietin levels, which can be considered another index of iron-restricted erythropoiesis in HF patients, being related to poor outcomes.[88,89]

Increased soluble transferrin receptor (sTfR) is another sensitive indicator of ID.[69,70,73,78,90–92] Soluble transferrin receptor is the

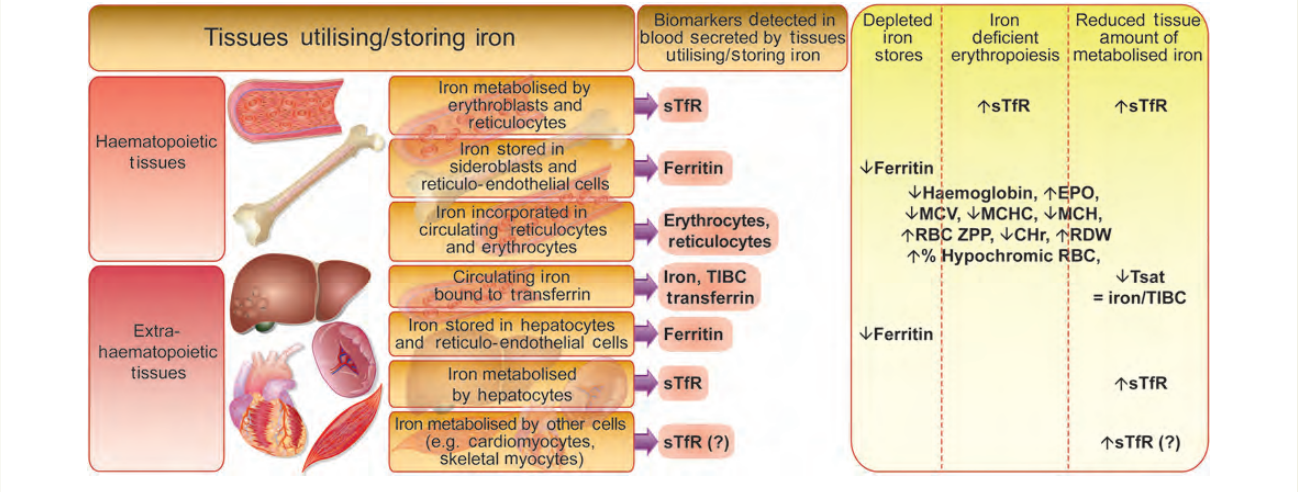

Figure 4 Tissues utilizing and/or storing iron and related biomarkers which are secreted by these tissues and can be detected in peripheral blood.

truncated form of transmembrane protein, a receptor for iron–transferrin complex and the major system responsible for the intracellular iron import.[56,57,73,90] It is present on virtually all cells, but a vast majority is localized on erythroid precursors.[57,90] When iron delivery to target tissues is insufficient for metabolic requirements, the expression of the transferrin receptor increases in order to facilitate intracellular iron influx.[56,57,70,73,90] Consequently, circulating sTfR (originating from all cells metabolizing iron) quantitatively reflects both the tissue iron demand (tissue iron balance) and the erythroid proliferation rate (total erythroblast mass), but not body iron stores.[69,70,73,78,90] No study has used this biomarker to indicate and/or guide therapy, and so it should be regarded as a research tool.

Because serum ferritin is a surrogate of iron stores and serum sTfR reflects the tissue iron demand, there is evidence that the combination of these two parameters may describe the iron status more accurately.[73,90,93]

Absolute and functional iron deficiency in heart failure

A pathophysiology milieu in HF syndrome favours the development of absolute and functional ID.

The following mechanisms are presumed to be involved in the development of absolute ID in HF: (i) insufficient dietary iron supply,[94,95] (ii) poor GI absorption, impaired duodenal iron transport,[96] drug interactions (e.g. omeprazole), or food reducing absorption, and (iii) GI blood loss (*Figure 3*).

Some studies demonstrate suboptimal dietary iron supply, particularly in patients with advanced HF. Based on a 4-day food diary, Hughes *et al.*[94] showed that 46% of patients with stable HF consumed less iron than the dietary reference value, and average daily iron intake was markedly reduced in patients in NYHA class III–IV when compared with NYHA class II. In another study, Lourenço *et al.*[95] assessed the nutritional status using an interview by nutritionists in 125 outpatients with stable HF, and in 12–35% found an inadequate dietary iron intake.

In HF, reduced iron intake may also be a consequence of deranged transport systems in the enterocytes. Theoretically, reduced expression of membrane proteins importing iron from the intestinal lumen to the enterocyte cytosol and the subsequent iron export to the circulation may result from increased circulating hepcidin levels, analogous to a reported experimental model of chronic kidney disease.[97,98] Recent experimental evidence demonstrates the existence of disrupted regulatory mechanisms of duodenal iron transportation systems in animals with induced HF and ID.[96] Animals from both HF and ID groups developed ID (and anaemia) along with a reduced hepatic expression of hepcidin compared with controls.[96] In animals with ID but without HF, there was up-regulation of the elements of the duodenal iron transportation system (duodenal cytochrome b, DMT-1, ferroportin), which was not seen in animals with ID and HF. More importantly, the intestinal expression of hypoxia-inducible factor-2α (the major regulator of the duodenal iron transportation system[98]) was up-regulated in iron-deficient animals without HF, but not in animals with HF.[96] This suggested a lack of adaptive physiological mechanisms to counteract depleted iron stores and to augment iron absorption in the duodenum.[96] These mechanisms have not been investigated in HF patients, and it remains unclear whether they would play any role in a clinical setting of HF.

Heart failure is a state characterized by generalized inflammation with an augmented immune response, overactive immune cells, high circulating levels of pro-inflammatory mediators, and the up-regulation of these molecules within the failing myocardium and peripheral tissues.[99–101] Activation of pro-inflammatory pathways constitutes an important element of the pathophysiology of HF, which triggers and maintains phenomena such as weight loss, impaired exercise capacity, insulin resistance, etc.[99,102–104] Hence, it is tempting to hypothesize that in HF, functional ID may be secondary to the inflammation, or due to inflammatory processes resulting from concomitant pathologies (e.g. renal failure, chronic infections).

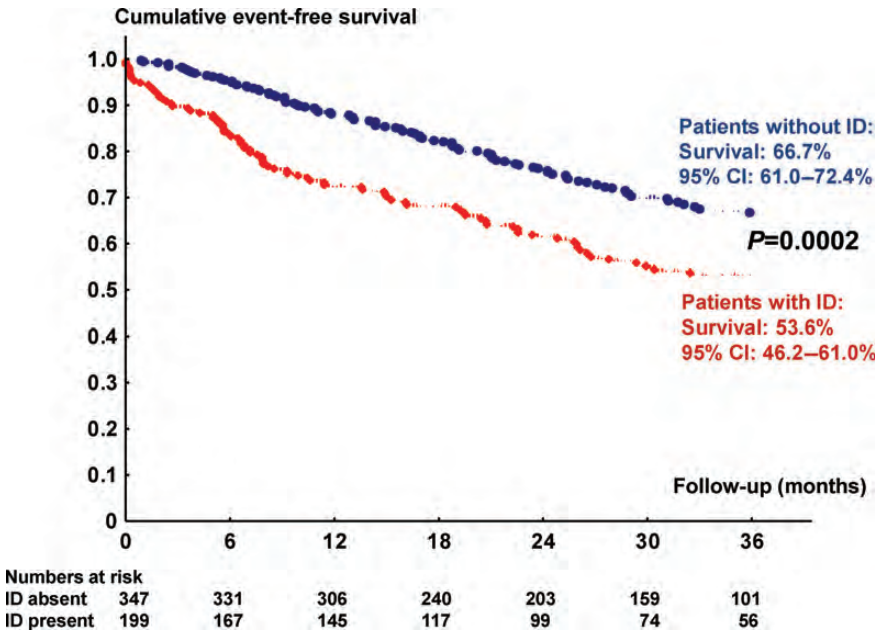

Figure 5 Kaplan–Meier curves reflecting 3-year event-free survival rates in 546 patients with systolic heart failure with vs. without iron deficiency.[112]

In this context, hepcidin can be expected to play an important role. Both in rodents[105] and humans,[106] acute myocardial ischaemia is accompanied by increased circulating hepcidin, which subsequently decreases during recovery. Simonis *et al.*[105] observed the parallel overexpression of hepcidin within the ischaemic and remote myocardium in rats. The role of hepcidin produced locally is unknown. Interestingly, in clinical settings of HF, there was no association between pro-inflammatory activation (as evidenced by circulating IL-6) and hepcidin levels.[107,108] Anaemic HF patients have reduced serum and urine hepcidin compared with non-anaemic and healthy subjects, which is accompanied by depleted total body iron.[107–109]

Incidence of iron deficiency in heart failure patients

Clinical evidence on the incidence of ID in HF patients is scarce. Most available studies have presented a traditional view linking ID with anaemia.[23–25,110] Additionally, difficulties in their interpretation are due to a lack of prospectively validated definition of ID in HF.

Ezekowitz *et al.*[23] provided the first evidence that ID frequently coexisted with anaemia in HF patients. In this study, anaemia was present in 17% of hospital discharges for HF, and ID was diagnosed in 21% cases of anaemia.[23]

Witte *et al.*[111] investigated the iron status in ambulatory patients with chronic HF using only the serum ferritin level. Iron deficiency (ferritin <30 μg/L) was found in 13% of HF patients, regardless of LVEF (functional ID not reported).[111]

Opasich *et al.*[25] examined 148 outpatients with systolic HF and concomitant anaemia, among whom 20% had microcytic anaemia that mainly reflected insufficient bone marrow iron utilization (absolute ID).[25] However, the commonest form was anaemia of chronic disease (57% of patients), and in this group nearly all demonstrated defective iron supply for erythropoiesis (functional ID).[25] The presence of ID was confirmed in 36% of all anaemic subjects and 64% of patients with anaemia of chronic disease.[25]

The only study assessing the iron status in HF patients based on the *gold standard* (bone marrow biopsy) was reported by Nanas *et al.*[24] Iron deficiency was confirmed in 27 (73%) of 37 anaemic patients with advanced decompensated HF.[24] Although, serum ferritin in ID subjects was lower compared with non-ID patients, the vast majority of ID patients had serum ferritin within the normal range,[24] further confirming the difficulty of evaluating ID in HF on the basis of serum ferritin assessment.

So far, only two observational studies have reported the incidence of ID in the general HF population.[110,112] Adlbrecht *et al.*[110] found ID (serum ferritin <30 μg/L or Tsat $<15\%$) in 26% of patients with chronic systolic HF, with an ID incidence of 16 and 54% in non-anaemic and anaemic subjects, respectively. We have demonstrated a 37% incidence of ID (serum ferritin <100 μg/L or serum ferritin 100–300 μg/L with Tsat $<20\%$) among 546 patients with chronic systolic HF.[112] The incidence of ID reached 32 and 57% in anaemic and non-anaemic patients, respectively.[112] We identified four independent determinants for a higher incidence of ID: female gender, advanced NYHA class, high plasma N-terminal pro-B-type natriuretic peptide (NT-pro-BNP), and high serum high-sensitivity C-reactive protein.[112] As we studied relatively young HF patients, predominantly men, in real life, the prevalence of ID may be even higher as HF patients are older, more frequently females, and with co-morbidities. Further studies are warranted.

Clinical and prognostic consequences of iron deficiency in heart failure patients

Iron deficiency and exercise intolerance in heart failure

In patients with stable systolic HF, ID was associated with reduced peak oxygen consumption and a high ventilatory response to exercise, also after an adjustment for clinical co-variables.[113] The difference in exercise capacity between iron-deficient and iron-replete subjects was seen separately in anaemics and non-anaemics.[113]

There is also indirect evidence linking correction of ID with an improvement in exercise capacity in a few interventional studies in HF patients, regardless of baseline anaemia.[80,81,114,115]

Iron deficiency and depression symptoms in heart failure

Iron deficiency carries also a risk of depression in men with systolic HF. Moderate depression by beck depression inventory (BDI) ($\geq$16 points) was more prevalent (48 vs. 25%), and the lack of depression symptoms (BDI $<$10 points) less common (13 vs. 51%) in men with ID than those without ID (E.A. Jankowska *et al.*, submitted for publication). Iron deficiency was associated with more severe depression symptoms, irrespective of HF severity, neurohormonal activation, haemoglobin, and inflammation (E.A. Jankowska *et al.*, submitted for publication).

Iron deficiency and prognosis in heart failure

The prognostic impact of ID in HF patients was investigated in only two observational prospective studies.[112,116] Varma *et al.*[116] investigated 120 consecutive patients with systolic dysfunction (LVEF $\leq$45%) undergoing percutaneous coronary intervention with a median follow-up of 30 months. They demonstrated that anaemia accompanied by ID strongly predicted cardiac mortality (33 vs. 1% in non-anaemics), malignancy-associated anaemia was related to high-non-cardiac mortality (57 vs. 4% in non-anaemics), whereas anaemia of chronic disease predicted neither cardiac nor non-cardiac death.[116] Among 546 patients with systolic HF we found that ID was a strong independent predictor of death and heart transplantation during a 3-year follow-up.[112] The presence of ID increased the risk of a poor outcome by 60% during the 3-year follow-up (*Figure 5*).[112]

Iron deficiency and dysfunction of myocardium and skeletal muscle

Mechanisms underlying links between ID and poor clinical status, exercise intolerance, and an unfavourable outcome in HF remain unclear. Dysfunction of both the myocardium and skeletal muscles are at the centre of the pathophysiology of HF.[99,117,118] These organs have high energy demands, and their function in dependent on intact iron metabolism.

Consequences of deranged iron metabolism for myocardium

Iron is an element of enzymes and structural proteins in cardiomyocytes, and is stored inside these cells. Molecular elements controlling iron metabolism are tracked within healthy, failing, ischaemic, and inflamed myocardium.[105,119–123] Hypoxia up-regulates hepcidin expression in the ischaemic rat myocardium (in contrast to hepatic hepcidin expression).[120] Rat cardiomyocytes from experimental myocarditis and myocardial infarction demonstrate increased hepcidin expression which normalizes 3 weeks after heart damage.[105,123] However, in the diseased myocardium, neither pathophysiological consequences of these changes nor their relationship with iron metabolism is understood.

Most available evidence reporting myocardial molecular consequences of ID comes from the experimental model of ID-anaemia. Iron deficiency-anaemic rats develop sympathetic activation with increased cardiac output,[14] left ventricular hypertrophy,[14–17,124–127] and finally left ventricular dilatation.[16,17] The myocardium from ID-anaemic rats is also characterized by the overexpression of ANP and BNP,[16] remodelling of extracellular matrix[16,128] and mitochondrial dysfunction.[17] In male rats with ID-anaemia and renal insufficiency, impaired left ventricular function was related to hypoferraemia and an increased semi-quantitative myocardial staining for hepcidin.[119] In this study, cardiomyocytes from hypertrophied hearts showed features of inflammation, hypoxia, apoptosis, and a local up-regulation of erythropoietin and hepcidin transcription when compared with tissues from sham-operated animals.[119] It can be concluded that in experimental models, anaemia and ID are accompanied by unfavourable changes in the myocardium.

Recently, Maeder *et al.*[120] demonstrated reduced iron content and reduced TfR 1 expression in failing human myocardium when compared with normal hearts. They provided experimental evidence that the myocardial expression of TfR 1 was regulated by β-adrenoceptor agonists and aldosterone.[129]

Consequences of deranged iron metabolism for skeletal muscle

Skeletal muscle accounts for 10–15% of the total body iron, and the system controlling iron metabolism is present there.[130–132] Sports medicine provided the earliest evidence linking ID and skeletal muscle function.[133–135] The optimal iron status in non-anaemic subjects was critical for the efficient increase in aerobic and endurance capacity with exercise training.[136–138]

The haemoglobin level and iron status are interlinked determinants of exercise capacity and physical fitness.[6] There are two determinants of exercise capacity and physical performance, i.e. tissue oxidative capacity and oxygen carrying capacity.[6] The former, which determines endurance, energy efficiency, and submaximal exercise effort, is mainly affected by the iron status. The tissue oxidative capacity is impaired proportionally across the whole spectrum of ID (also when haemoglobin is normal).[6] In contrast, the oxygen-carrying capacity determines mainly the aerobic capacity and the maximal exercise effort. The oxygen capacity is limited only with the most severe ID, when erythropoiesis is compromized with reduced haemoglobin.[6]

Table 1 Summary of seven studies with intravenous iron therapy administered in patients with heart failure

Publication	Studied groups		Iron therapy				Major results				
	Inclusion criteria: clinical status	Inclusion criteria: Hb and iron status	Study design	Iron preparation	Dose	Period	Hb and iron status	QoL, HF symptoms	Exercise capacity	CV events	Others
Bolger *et al.*[115]	$n = 16$, systolic HF, NYHA II–III	Hb ≤12 g/dL Ferritin ≤400 μg/L	Open-label, uncontrolled, single-centre	Iron sucrose	Maximum 1000 mg iron i.v. during 17 days (200 mg i.v. iron on Days 1, 3, 5, and if ferritin <400 μg/L on Day 12, also 200 mg i.v. iron on Days 15, 17)	12–17 days of therapy and further follow-up up to 3 months	↑ Hb ↑ Ferritin, ↑ Tsat	↑ QoL	↑ 6MWD		↓ (trend) cystatin C
Toblli *et al.*[114]	$n = 40$, LVEF ≤35%, NYHA II-IV, creatinine clearance ≤90 mL/min	Hb <12.5 g/dL for men, Hb <11.5 g/dL for women Ferritin <100 μg/L or Tsat ≤20%	Radomized, double-blind, placebo-controlled, single-centre	Iron sucrose vs. placebo (20 vs. 20)	200 mg iron i.v. weekly for 5 weeks Total iron dose: 1000 mg	5 weeks of therapy and follow-up up to 6 months	↑ Hb ↑ Ferritin, ↑ Tsat	↑ QoL, ↓ NYHA class	↑ 6MWD	↓ Hospitalization rate	↑ Creatinine clearance, ↑ LVEF, ↓ plasma NT-pro-BNP, ↓ CRP, ↓ resting heart rate
Okonko *et al.*[80] (FERRIC-HF)	$n = 35$, NYHA class II–III, peak VO_2 ≤18 mL/min/kg, LVEF ≤45%	Hb <12.5 g/dL (anaemic group) or Hb 12.5–14.5 g/dL (non-anaemic group) Ferritin <100 μg/L, or ferritin 100–300 μg/L and Tsat <20%	Randomized, open-label, observer-blinded, placebo-controlled, double centre	Iron sucrose vs. placebo (24 vs. 11)	Correction phase: 200 mg iron i.v. weekly until ferritin ≥500 μg/L Maintenance phase: 200 mg iron i.v. every 4 weeks Iron repletion total dose: estimated using Ganzoni formula	16 weeks of therapy and final assessments after next 2 weeks	All: ↑ ferritin, ↑ Tsat, ↑ Hb Anaemics: ↑ ferritin, ↑ Tsat, ↔Hb Non-anaemics: ↑ ferritin, ↑ Tsat, ↔ Hb	All: ↑ PGA, ↓ NYHA class, ↑ QoL Anaemics: ↓ NYHA class Non-anaemics: ↓ (trend) NYHA class,	All: ↑ (trend) peak VO_2 (mL/min), ↑ peak VO_2 (mL/min/kg) Anaemics: ↑ peak VO_2 (mL/min), ↑ peak VO_2 (mL/min/kg) Non-anaemics: ↔ peak VO_2 (mL/min), ↔ peak VO_2 (mL/min/kg)		
Usmanov *et al.*[146]	$n = 32$, NYHA III–IV, moderate renal failure (mean serum creatinine: 2.3 mg/dL)	Hb <11 g/dL Ferritin not specified	Open-label, uncontrolled, single-centre	Iron sucrose	Correction phase: 100 mg iron i.v. three times weekly for 3 weeks Maintenance phase: 100 mg iron i.v. weekly for 23 weeks Total iron dose: 3200 mg	26 weeks	NYHA III: ↑ Hb NYHA IV: ↑ Hb	NYHA III: ↓ NYHA class NYHA IV: ↔ NYHA class			NYHA III: ↓ PWT, ↓ ST, ↓ LVEDD, ↓ LVEDV, ↓ LVESD, ↓ LVESV, ↓ LV mass index, ↑ LVEF NYHA IV: ↓ PWT, ↔ ST, ↓ LVEDD, ↓ LVEDV, ↓ LVESD, ↓ LVESV,↓ LV mass index, ↔ LVEF
Drakos *et al.*[145]	$n = 16$	Anaemia (+) Iron deficiency (+) verified by bone marrow aspiration	Randomized, open label, single-centre	Iron sucrose vs. iron sucrose + darbapoietin α (8 vs. 8)	IV iron (300 mg weekly) vs. IV iron (300 mg weekly + darbapoietin α (50 μg sc weekly)	6 weeks of therapy and further 6 weeks of follow-up	↑ Hb (in absolute units and in %, similar in both arms)				
Comín-Colet *et al.*[144]	$n = 65$, NYHA class III–IV mild to moderate chronic kidney disease (stage II–IV) or serum creatinine <3 mg/dL	Hb <13 g/dL for men, Hb <12 g/dL for women Ferritin not specified	Open-label, uncontrolled, single-centre	Recombined human erythropoietin (rhuEPO) + iron sucrose vs. none (27 vs. 38)	rhuEPO—sc 4000 U per week, doses adjusted according to target Hb 12.5–14.5 g/dL IV iron 200 mg per week for 5–6 weeks, later 200 mg every 4–6 weeks (adjusted according to haematinics)	15 ± 9 months	↑ Hb ↔ Ferritin, ↑ Tsat,			↓ Number of CV admissions, ↓ days spent in hospital for CV causes, ↓ CV hospitalization rate, ↔ all-cause-mortality	↓ Plasma NT-pro-BNP,

Continued

Table 1 *Continued*

Publication	Studied groups	Iron therapy				Major results				
	Inclusion criteria: clinical status	Inclusion criteria: Hb and iron status	Study design	Iron preparation	Dose	Period	Hb and iron status	QoL, HF symptoms	Exercise capacity	CV events	Others
Anker *et al.*[81] (FAIR-HF)	$n = 459$, LVEF ≤40% and NYHA II, LVEF ≤45% and NYHA III	Hb: 9.5–13.5 g/dL Ferritin <100 μg/L or ferritin 100–300 μg/L and Tsat <20%	Randomized (2:1), double blind, placebo controlled, multicentre	Ferric carboxymaltose vs. placebo (304 vs. 155)	Correction phase: 200 mg i.v. iron week until repletion dose is achieved Maintenance phase: 200 mg i.v. iron every 4 weeks Iron repletion total dose: estimated using Ganzoni formula	24 weeks	↑ Hb ↑ Ferritin, ↑ Tsat, ↑ MCV (all patients, and separately: anaemics and non-anaemics)	↑ PGA, ↓ NYHA class (all patients, and separately: anaemics and non-anaemics)	↑ 6MWD	↓ (trend) CV hospitalization rate	

Hb, haemoglobin; QoL, quality of life; HF, heart failure; CV, cardiovascular; NYHA, New York Heart Association; i.v., intravenous; Tsat, transferrin saturation; 6MWD, 6-minute walking distance; PWT, posterior wall thickness; ST, septal thickness; LV, left ventricular; LVEDD, left ventricular end diastolic diameter; LVEDV, left ventricular end diastolic volume; LVESD, left ventricular end systolic diameter; LVESV, left ventricular end systolic volume; LVEF, left ventricular ejection fraction; PGA, patient global assessment; VO_2, oxygen consumption; FERRIC-HF, Ferric Iron Sucrose in Heart Failure; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; CRP, C-reactive protein; FAIR-HF, Ferinject Assessment in Patients with Iron Deficiency and Chronic Heart Failure; MCV, mean corpuscular volume.

In rodent studies, the distinctions between the effects of diminished oxygen transport and oxygen diffusion and decreased oxidative capacity (due to ID at the tissue level, not necessarily linked with anaemia) have been established both in resting and exercising skeletal muscles.[133,134,139] Additionally, impaired bioenergetics and abnormal patterns of glucose and free-fatty acid utilization as fuel sources with earlier lactate accumulation in exercising muscles at submaximal exercise in ID animals have been described.[140,141] Finch *et al.*[142] investigated ID anaemic rats who received different combinations of blood transfusion and/or iron-rich diet in order to obtain a similar increase in the haemoglobin level at different levels of iron repletion. An improvement in exercise capacity was not directly related to an increase in haemoglobin, but exercise capacity increased only in animals who received iron supplementation.[142] Iron administration in ID non-anaemic young subjects increased serum ferritin (but not haemoglobin) and improved the submaximal energy efficiency.[143]

Almost all available evidence linking the iron status with skeletal muscle function comes from physiological experiments and studies performed in healthy subjects. It remains unclear whether analogous mechanisms may explain the unfavourable effects of ID on exercise capacity in HF patients. Comprehensive studies are needed in this field.

Iron supplementation in patients with heart failure

The effects of i.v. iron supplementation in HF patients were reported in seven studies: three open-label uncontrolled studies,[115,143,144] two randomized open-label studies,[80,145] two randomized double-blind placebo-controlled trials.[81,113] Among them only two included both anaemic and non-anaemic HF patients[80,81] (details in *Table 1*).

The first study by Bolger *et al.*[115] provided data on 16 cases that iron sucrose given i.v. for 5–17 days in anaemic ID HF patients was well tolerated, increased haemoglobin, and improved symptoms and exercise capacity over a 3-month follow-up period. Toblli *et al.*[114] confirmed in the first controlled study that i.v. iron treatment in anaemic HF patients with impaired renal function improved the functional status, exercise capacity, and quality of life. They also reported other beneficial effects of iron therapy on LVEF, plasma NT-pro-BNP and CRP, and hospitalization rate,[114] but the small numbers make these findings uncertain.

In the FERRIC-HF (*FERRIC I*ron *S*ucrose in *H*eart *F*ailure) study,[80] 16 weeks of i.v. iron therapy was well tolerated, and improved exercise tolerance and symptoms. Interestingly, benefits were also observed in non-anaemic ID patients although to a lesser extent, and an increase in the peak oxygen consumption was not related to changes in haemoglobin, but to an increment in the Tsat.[80]

Usmanov *et al.*[146] demonstrated that i.v. iron given for 26 weeks to patients with advanced HF, anaemia, and chronic renal insufficiency exerted favourable anti-remodelling effects on the myocardium assessed by echocardiography, and improved the functional class (only in NYHA class III patients). In the study by Drakos *et al.*[145] i.v. iron supplementation with erythropoietin in HF patients with anaemia and ID, verified by bone marrow aspiration,

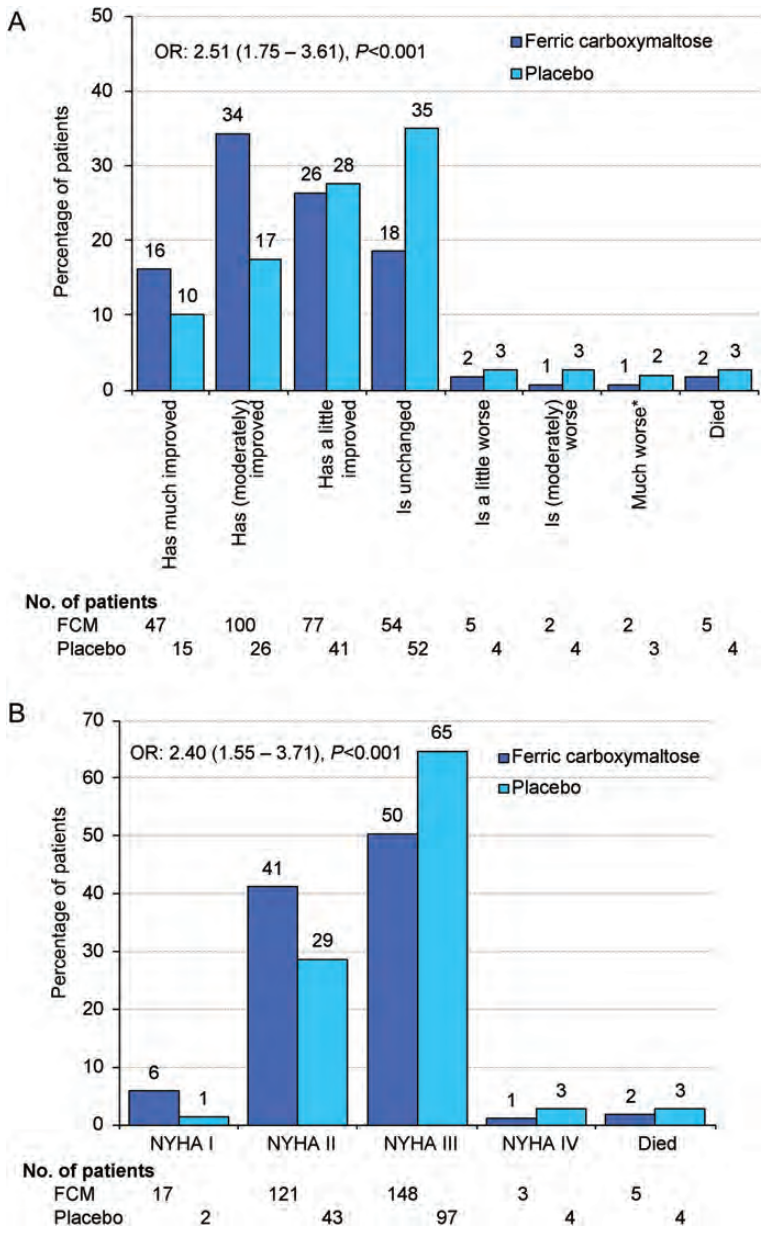

Figure 6 Self-reported Patient Global Assessment and NYHA functional class at week 24 (primary endpoints of FAIR-HF trial), according to assigned study treatment (intravenous iron vs. placebo).[81]

increased haemoglobin to a similar extent to erythropoietin alone. Comín-Colet *et al.*[144] reported that long-term therapy with i.v. iron and erythropoietin in elderly patients with advanced HF, renal dysfunction, and anaemia, and corrected haemoglobin and creatinine levels, improved symptoms and decreased plasma NT-pro-BNP. This therapy was also associated with an 80% reduction in the combined endpoint of all-cause mortality and cardiovascular hospitalizations.[144]

FAIR-HF (*F*erinject® Assessment in patients with *I*Ron deficiency and chronic *H*eart *F*ailure) study was a randomized double-blind placebo-controlled multi-centre trial, which so far recruited the greatest number of patients with chronic systolic HF and ID (both anaemics and non-anaemics) ($n = 459$) who subsequently received a 24-week therapy of i.v. iron or placebo (2:1).[81] Beneficial effects of i.v. iron therapy on the NYHA class and the patient's global assessment were seen across the whole clinical spectrum of HF (*Figure 6*) (regardless of the baseline NYHA class, haemoglobin, LVEF, HF aetiology, the presence of co-morbidities).[81] There was no increased risk of side-effects in the treated vs. the non-treated group, but the observation was limited to 6 months.[81] Although the FAIR-HF trial was not designed to test the effects of iron therapy on the outcome, the authors reported a trend towards a reduced rate for the first cardiovascular hospitalization in the treated vs. the non-treated group,[81] which is similar to other

reports.[114,144] Undoubtedly, there is a need for more and longer-running, randomized, double-blind, placebo-controlled trials that could validate the findings of FAIR-HF and also investigate the impact of this novel treatment modality on the morbidity and mortality in HF patients with ID.

Conclusions

Iron is a micronutrient that stands at the centre of cellular metabolism and is critical for the maintenance of homoeostasis.

Iron deficiency constitutes a frequent co-morbidity in HF patients. Iron deficiency is gaining interest, not only as an aetiological factor leading to and/or aggravating anaemia in HF, but is considered a separate condition with unfavourable clinical and prognostic consequences. There is experimental evidence suggesting that iron supplementation in iron-deficient animals may activate molecular pathways protecting the heart and preventing myocardial remodelling. Only recently, clinical studies demonstrated that in HF patients with ID, i.v. iron repletion was well-tolerated, and improved functional status, quality of life, and exercise capacity.

There are the premises that HF patients may benefit from the correction of anaemia, ID, or both. It is emphasized that currently there is neither convincing nor unequivocal evidence on the most accurate intervention to be applied in the two conditions. This is partially due to the unclear pathophysiology of ID in HF as well as lack of a clinically applicable and prospectively verified definition of this condition, all of which justify a need for future mechanistic and interventional studies. Further studies will finally establish whether ID may become a novel therapeutic target in HF patients.

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