6 Definition of iron deficiency based on the gold standard of bone marrow iron staining in heart failure patients.

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ABSTRACT

Background

The most commonly used definition of iron deficiency (ID) (ferritin<100ng/mL or ferritin 100-300ng/mL and transferrin saturation [TSAT]<20%) has not been validated in patients with heart failure (HF). We aimed to define and validate the biomarker-based definition of ID in HF, using bone marrow iron staining as the gold standard. Second, we aimed to assess the prognostic value of the optimized definition.

Methods and Results

Bone marrow aspiration with iron staining was performed in 42 patients with HF and a reduced ejection fraction (LVEF≤45%) undergoing median sternotomy for coronary artery bypass grafting. Patients were mostly male (76%) with mild to moderate HF and a mean age of 68±10 years. Bone marrow ID was found in 17 (40%) of the HF patients. The most commonly used definition of ID had a sensitivity of 82% and a specificity of 72%. A definition solely based on TSAT≤19.8% or serum iron≤13µmol/L had a sensitivity of 94 and specificity of 84% and 88% respectively ($p<0.05$ compared to the former definition). Subsequently, we assessed the incidence of all-cause mortality in 387 consecutive outpatient HF patients (LVEF≤45%). In these patients, TSAT≤19.8% and serum iron≤13µmol/L, and not ferritin, were independently associated with mortality.

Conclusions

A TSAT≤19.8% or a serum iron≤13µmol/L show the best performance in selecting patients with ID and indentifies HF patients at the highest risk of death. Our findings validate the currently used TSAT cut-off of <20% for the identification of ID in HF patients, but question the diagnostic value of ferritin.

INTRODUCTION

Iron deficiency (ID), either with or without anemia, is an important co-morbidity in heart failure (HF) patients.[1–4] ID limits aerobic performance and exercise tolerance and is associated with a worse prognosis.[1–3] Correction of ID with intravenous iron therapy improves symptoms, quality of life and functional capacity and a recent meta-analysis of four randomized trials showed an association between administration of intravenous ferric carboxymaltose (FCM) and a reduction of cardiovascular hospitalizations and cardiovascular mortality.[5–8]

Diagnosing ID in daily practice is based on circulating biomarkers including ferritin, iron and transferrin saturation (TSAT). Because ferritin is an acute phase reactant, levels tend to rise in inflammatory conditions.[9] This implies that a correction of diagnostic cut-off values is necessary for patients with HF compared to the general population, as low-grade inflammation frequently accompanies HF.[10] This correction is made for ferritin (the commonly used cut-off for ferritin is between 12 – 40ng/mL[11], but has – arbitrarily – been set to <100 ng/mL in HF patients) and applied in combination with TSAT (TSAT <20% if ferritin 100-300 ng/mL). This definition has been used in several studies that tested the value of administration of intravenous iron.[6,7,12] However, these cut-offs have never been validated using the gold standard: bone marrow iron staining. Clinical trials in ID might therefore target a group of patients that may not all have true ID.

To study the true prevalence of ID and to identify the optimal circulating biomarkers and cut-off values for the diagnosis of ID in patients with HF, we compared a wide range of hematological and iron markers with bone marrow iron staining, the gold standard for ID diagnosis. Subsequently, we assessed prognostic associations of the optimized definition in outpatient HF patients.

MATERIAL AND METHODS

The data and study materials will be made available to other researchers upon request for purposes of reproducing the results or replicating the procedure.

Patients

Bone marrow study

We studied patients who were scheduled for coronary artery bypass graft (CABG) surgery at the University Medical Center Groningen, Groningen, The Netherlands with a

history of HF with a NT-proBNP of >125 pg/mL and reduced left ventricular ejection fraction (LVEF ≤45%) assessed by an echocardiogram (N=49) or multi gated acquisition scan (N=1). Exclusion criteria were a history of acquired iron overload, iron therapy in the previous year or any disease known to possibly influence iron metabolism, such as severe renal failure (estimated glomerular filtration rate [eGFR] <30 ml/min/1,73m^2), infection, hematological disease, malignancy, hepatic disease or a systemic inflammatory disease such as vasculitis or rheumatoid arthritis. In total, 50 patients were included in the study but data were incomplete in 8 cases (2 patients did not undergo surgery and 6 failed bone marrow assessments because of too little material).

The study protocol was approved by the local ethics committee and the study was conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent prior to any study-related procedures.

Outpatient HF clinic

HF patients who visited our tertiary referral academic hospital regarding follow-up after HF admission were used in this study. A total of 640 consecutive outpatient HF patients, diagnosed according to the ESC guidelines, were included in the registry between February 2014 and March 2016.[13] As a part of the standard work-up, we performed assessment of left ventricular function with echocardiography, biochemical analyses, recording of medication use and follow-up. Follow-up consisted of all-cause mortality and data were verified using the 'Municipal Personal Records Database' register. Patients were optimally treated according to ESC guidelines, with ACE-inhibitors or ARBs, beta blockers, and mineralocorticoid receptor antagonists, unless not tolerated or contraindicated, and received devices when indicated.[13] Patients were excluded from these analyses if ferritin or transferrin saturation levels were unknown (N=55), LVEF >45% (N=164) or patients received intravenous or oral iron therapy (N=34), resulting in 387 patients available for the current analysis.

Bone marrow assessment

Bone marrow aspirates were taken from the sternum in patients with HF during CABG, just before median sternotomy was performed. In a certified core-lab, the Prussian blue staining with potassium ferrocyanide was used on multiple slides per sample to assess the presence of non-heme bound iron in the erythroblasts and the extracellular space. All slides were assessed by two independent analysts. The percentage of erythroblasts containing iron, i.e. sideroblasts, reflects the amount of iron incorporated in the erythrocyte precursor cells and thus the functional availability of iron for erythropoiesis.[14] In normal conditions, 20-50% of the erythroblasts contain iron, 10-20% is considered low normal, and patients with sideroblasts <10% are considered functionally iron deficient.[15]

The iron stores are assessed as the amount of iron present in the extracellular space and graded using Gale's histological grading method.[16] Bone marrow with grade zero (no iron) or grade one (trace of iron, just visible under high power magnification [x1000]) is considered as "iron storage depleted". Using the information regarding stores and erythroblast incorporation, both functional ID (normal stores, impaired incorporation) and absolute ID (impaired stores and incorporation) were detected, together these were classified as ID.[17]

Analytical methods

Fresh venous blood with ethylenediaminetetraacetic acid was used to measure hematological parameters. The hematological profile was analyzed using the Sysmex XN20 (Sysmex Corporation, Kobe, Japan), and included the following parameters: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, reticulocyte hemoglobin content, red cell hemoglobin content and the derived absolute difference between the hemoglobin content of the reticulocyte and red cell, reticulocyte count, white blood cell count and the percentage of hypochromic red cells.

Markers of iron status assessed using standard methods on a Roche modular cobas 8000 (Roche Diagnostics, Indianapolis, USA) included: serum ferritin, serum iron and serum transferrin. Serum soluble transferrin receptor levels were measured using immunonephelometry on a BNII Nephelometer (Siemens AG, Erlangen, Germany) and serum hepcidin levels were measured using a competitive enzyme-linked immunosorbent assay, as described previously.[18] TSAT is the percentage of transferrin saturated with iron and was calculated using serum iron and serum transferrin using the following formula: TSAT (%) = iron (µmol/L) / (transferrin [g/L] x 25.2) x 100.[19] Serum C-reactive protein (CRP) and other blood markers were assessed using standard methods. All laboratory measurements were done in fresh venous blood except for serum soluble transferrin receptor and hepcidin. These were measured in serum stored at -80°C for an average time of 12 months which was never thawed before assaying.

Other clinical parameters

Anemia was defined according to the World Health Organization criteria as a hemoglobin level <13.0 g/dl in men and <12.0 g/dl in women.[20] The reticulocyte production index was calculated as follows: (reticulocytes * (hematocrit /0.45)) / maturation correction. The maturation correction reflects the longer lifespan of prematurely released reticulocytes in case of a low hematocrit varying from 1.0 days at a hematocrit of 0.36 to 0.45, to 2.5 days at a hematocrit <0.15. The serum soluble transferrin receptor-ferritin index was calculated as the ratio between serum soluble transferrin receptor and log

transformed ferritin levels.[21] Diabetes mellitus was considered present when a subject was on antidiabetic medication or had a glycated hemoglobin ≥48 mmol/mol. The glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula based on serum creatinine levels.[22]

Hypercholesterolemia was defined as total serum cholesterol ≥5.0 mmol/L (193 mg/dL), or when lipid-lowering medication was used. Hypertension was considered present when a subject had a systolic blood pressure >140 mmHg, a diastolic blood pressure >90 mmHg or when he or she had a history of hypertension.

Statistical analyses

Data are presented as means ± standard deviation when normally distributed, as medians and interquartile range when non-normally distributed, or as frequencies and percentages for categorical variables. Differences between baseline variables were tested using the one-way analysis of variance test, Wilxocon rank-sum (2 groups) and Kruskal-Wallis test (3 groups) and Pearson's χ^2 test, respectively.

Receiver operator characteristic (ROC) curve analysis was performed to estimate the ability of the different markers of iron status to predict bone marrow iron stores and availability. The area under the curve (AUC) reflects the performance of the test with a score >0.80 considered a good accuracy and >0.70 is considered to be fair. The optimal cut-off value is defined as the value with the minimal distance of the ROC curve to the upper left corner: $d^2=(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$. All biomarker test with a good accuracy (AUC >0.80) and those previously identified in the literature were dichotomized using this optimal cut-off and compared to the FAIR-HF (Ferinject Assessment in Patients With Iron Deficiency and Chronic Heart Failure) definition with regard to AUC's, sensitivity and specificity. Differences between AUC's and sensitivity/specificity were tested using the DeLong test and Mc-Nemar test, respectively.[23]

In the outpatient HF patients, Cox proportional hazard regression analyses on all-cause mortality were performed univariable and in a multivariable model including all variables included in the MAGGIC risk score (Meta-Analysis Global Group in Chronic Heart Failure) (except smoking status and time since diagnosis due to unavailability of the data) and additionally corrected for serum sodium, hemoglobin and log transformed CRP.[24] Cumulative incidence curves were constructed to estimate incidence of new onset HF and the log-rank test was used to compare the incidence curves. Follow-up was truncated when <5% of the subjects were at risk, which was at 746 days.

We considered a two-sided P-value of < 0.05 statistically significant. All tests and analyses were performed using STATA version 13.0 (StataCorp LP, College Station, Texas, USA).

RESULTS

Patients characteristics

Baseline characteristics of the 42 HF patients, stratified for bone marrow iron status, are presented in **Table 1**. Mean age was 68±10 years, 76% of the patients were male, LVEF was 38±7%, NT-proBNP 914 (454–1755) ng/L and the majority of patients were in NYHA class II or III (50% and 29% respectively). Forty percent of the subjects had ID based on the gold standard (<10% of bone marrow erythroblasts containing iron, with or without low iron stores). Clinical characteristics did not significantly differ between subjects with and without ID, although a history of atrial fibrillation was more prevalent in patients without ID. Patients with ID had higher levels of the inflammatory parameters CRP and erythrocyte sedimentation rate, and a higher glycated hemoglobin level. Additionally, none of the patients used erythropoiesis-stimulating agents.

Table 1 – Baseline characteristics.

Variable	Total	Normal BM iron	Iron deficiency	P-value*
N	42	25	17	
Age, y	68.0 ± 9.5	67.4 ± 9.6	68.8 ± 9.7	0.65
Female gender	10 (24%)	5 (20%)	5 (29%)	0.48
BMI, kg/m2	28.6 ± 3.8	28.6 ± 3.4	28.8 ± 4.6	0.88
SBP (mmHg)	131.5 ± 16.5	132.2 ± 14.8	130.4 ± 19.2	0.73
NYHA class				0.37
1	8 (19%)	6 (24%)	2 (12%)	
2	21 (50%)	13 (52%)	8 (47%)	
3	12 (29%)	5 (20%)	7 (41%)	
4	1 (2%)	1 (4%)	0 (0%)	
LVEF, %	37.8 ± 7.0	38.9 ± 7.3	36.3 ± 6.4	0.24
HF diagnosed <90 days	19 (45%)	10 (40%)	9 (53%)	0.41
Comorbidities				
Previous MI	20 (48%)	9 (36%)	11 (65%)	0.067
Diabetes mellitus	22 (52%)	10 (40%)	12 (71%)	0.051
Atrial fibrillation	12 (29%)	10 (40%)	2 (12%)	0.047
Hypertension	32 (76%)	20 (80%)	12 (71%)	0.48
Hypercholesterolemia	39 (93%)	24 (96%)	15 (88%)	0.34
ID (FAIR-HF)	21 (50%)	7 (28%)	14 (82%)	<0.001
Anemia	7 (17%)	2 (8%)	5 (29%)	0.068

Table 1 – Baseline characteristics. (*continued*)

Variable	Total	Normal BM iron	Iron deficiency	P-value*
Laboratory values				
NT-proBNP, ng/L	914 (454, 1755)	718 (436, 1749)	1234 (529, 2050)	0.40
eGFR, mL/min/1.73m2	77.9 ± 18.8	78.8 ± 15.4	76.7 ± 23.3	0.72
Sodium, mmol/L	139.8 ± 3.0	140.0 ± 3.1	139.4 ± 3.1	0.52
LDH, U/l	175 (163, 191)	174 (163, 188)	179 (155, 204)	0.85
CRP, mg/L	2.0 (0.9, 4.5)	1.5 (0.7, 2.1)	3.0 (1.8, 10.0)	0.020
ESR, mm/hour	14 (4, 32)	8 (3, 18)	34 (16, 42)	<0.001
HbA1c, %	6.3 (5.7 – 7.0)	5.8 (5.6 – 6.6)	6.5 (6.2 – 7.4)	0.014
HDL/LDL ratio	0.48 (0.36, 0.62)	0.48 (0.36, 0.59)	0.47 (0.39, 0.62)	0.86
AST, U/l	22 (19, 27)	24 (20, 27)	21 (19, 23)	0.36
ALT, U/l	20 (16, 24)	20 (17, 26)	19 (14, 21)	0.054
Hematology				
Hemoglobin, g/dL	14.0 ± 1.3	14.6 ± 1.1	13.1 ± 1.1	<0.001
Hematocrit, %	0.42 ± 0.03	0.43 ± 0.03	0.40 ± 0.03	0.006
Reticulocytes, ‰	13.2 ± 4.3	12.6 ± 4.1	14.1 ± 4.6	0.28
RPI	56.4 ± 18.3	59.4 ± 18.7	52.1 ± 17.3	0.21
RDW, %	13.7 ± 1.8	13.1 ± 0.9	14.6 ± 2.4	0.007
MCV, fl	90.1 ± 5.3	91.1 ± 5.1	88.6 ± 5.4	0.13
MCH, fmol	1881 ± 151	1931 ± 127	1806 ± 156	0.008
MCHC, g/dL	20.9 ± 0.8	21.2 ± 0.6	20.4 ± 0.9	0.001
Iron, µmol/L	15 (9 – 19)	18 (15 – 21)	9 (7 – 10)	<0.001
Ferritin, ng/mL	144 (85, 263)	159 (107, 271)	104 (44, 162)	0.071
TSAT, %	20.9 (14.7, 27.8)	27.5 (21.3, 31.7)	14.3 (11.6, 17.0)	<0.001
Transferrin, mg/dL	258.8 ± 43.3	256.0 ± 38.5	262.9 ± 50.6	0.62
HYPO, %	0.1 (0.1, 0.2)	0.1 (0.1, 0.1)	0.2 (0.1, 0.5)	0.037
RET-He, pg	32.1 ± 2.6	33.2 ± 1.6	30.6 ± 2.9	<0.001
RBC-He, pg	29.9 ± 2.3	30.6 ± 1.7	28.8 ± 2.7	0.011
Delta-He, pg	2.2 ± 0.8	2.6 ± 0.7	1.8 ± 0.7	0.002
sTfR, mg/L	1.09 (0.94, 1.42)	1.05 (0.92, 1.24)	1.16 (1.02, 1.60)	0.051
sTfR-F index	0.15 (0.13, 0.19)	0.15 (0.13, 0.17)	0.19 (0.15, 0.34)	0.025
Hepcidin, nM	10.8 (5.9 – 15.8)	11.4 (7.1 – 13.9)	6.1 (1.2 – 28.2)	0.65
Medication				
Anti-platelet therapy	33 (79%)	17 (68%)	16 (94%)	0.043
Diuretics	22 (52%)	14 (56%)	8 (47%)	0.57

Table 1 – Baseline characteristics. (*continued*)

Variable	Total	Normal BM iron	Iron deficiency	P-value*
β-blocker	32 (76%)	21 (84%)	11 (65%)	0.15
ACEi or ARB	38 (90%)	23 (92%)	15 (88%)	0.68
MRA	12 (29%)	5 (20%)	7 (41%)	0.14
OAC	10 (24%)	8 (32%)	2 (12%)	0.13

* Normal vs. iron deficient patients. Data are presented as mean ± standard deviation when normally distributed, as median and interquartile range when non-normally distributed, or as frequencies and percentages for categorical variables. BMI=body mass index; SBP=systolic blood pressure; NYHA class=New York Heart Association class; LVEF=left ventricular ejection fraction; MI=myocardial infarction; ID=iron deficiency; eGFR=estimated glomerular filtration rate; LDH=lactate dehydrogenase; CRP=c-reactive protein; ESR=erythrocyte sedimentation rate; HDL=high density lipoprotein; LDL=low density lipoprotein; AST=aspartate transferase; ALT=alanine transferase; RPI=reticulocyte production index; RDW=red blood cell distribution width; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; TSAT=transferrin saturation; HYPO=hypochromic red blood cells; RET-He=reticulocyte hemoglobin content; RBC-He=red blood cell hemoglobin content; Delta-He=difference between RBC-He and RET-He; sTfR=soluble transferrin receptor; sTfR-F index=ratio between sTfR and log transformed ferritin; ACEi=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker, MRA=mineralocorticoid receptor antagonists, OAC=oral anticoagulants.

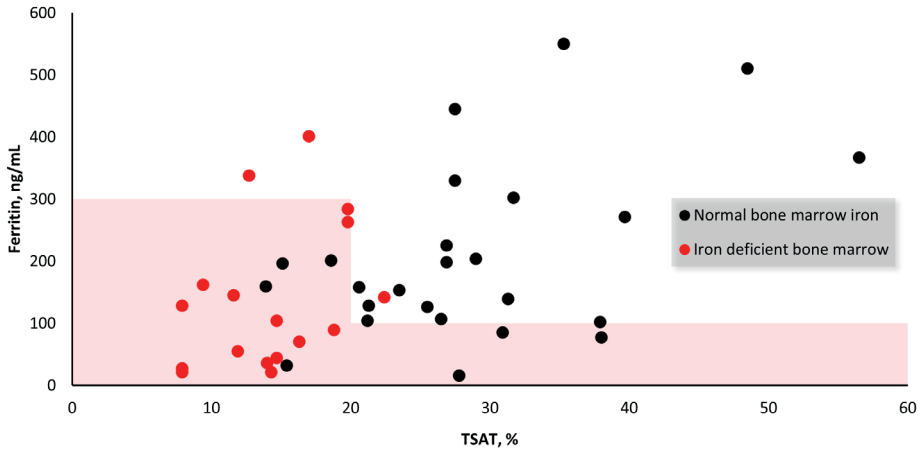

Figure 1 – Ferritin and TSAT levels compared to bone marrow iron status. Each dot represents one patient with the black dots reflecting normal bone marrow iron status and the red dots iron deficient patients. The red colored area represents the FAIR-HF definition (ferritin <100 ng/mL or ferritin 100-300 ng/mL with a TSAT <20%). TSAT=transferrin saturation.

Diagnostic accuracy of serum markers

Results of bone marrow defined ID, either functional or absolute, versus ferritin and TSAT values are depicted in **Figure 1**. The definition used in the clinical trials is displayed as the red colored area. Most patients with bone marrow defined ID fall into the area of

TSAT ≤19.8%, while the use of a combination of a low ferritin with a TSAT>19.8% does not identify patients with bone marrow defined ID. This is confirmed by the results of the ROC analysis (**Table 2**), which shows TSAT ≤19.8% to be the best diagnostic cut-off together with serum iron ≤13µmol/L with AUCs of 0.932 and 0.922, respectively. Nota-

Table 2 – Receiver operating characteristics for the presence of ID.

Variables for prediction of ID	AUC ± SE	95% CI	Cut-off value	Sensitivity	Specificity
Hemoglobin, g/dL	0.820 ± 0.064	0.696 – 0.944	≤14.2	94.1%	48.0%
Hematocrit, %	0.716 ± 0.081	0.558 – 0.874	≤0.41	70.6%	58.3%
Reticulocytes, x10^9/L	0.586 ± 0.095	0.399 – 0.772	≥13.1	64.7%	58.3%
RPI	0.618 ± 0.091	0.439 – 0.796	≤60.2	82.4%	50.0%
MCV, fl	0.645 ± 0.090	0.469 – 0.821	≤90.1	76.5%	62.5%
MCH, fmol	0.719 ± 0.084	0.554 – 0.883	≤1879	75.0%	66.7%
MCHC, g/dL	0.773 ± 0.080	0.618 – 0.929	≤20.9	75.0%	66.7%
RDW, %	0.733 ± 0.083	0.570 – 0.895	≥13.5	58.8%	75.0%
HYPO, %	0.687 ± 0.091	0.509 – 0.865	≥0.2	64.7%	78.3%
RET-He, pg	0.821 ± 0.066	0.692 – 0.950	≤32.2	76.5%	73.9%
RBC-He, pg	0.706 ± 0.086	0.536 – 0.875	≤30.0	82.4%	69.6%
Delta-He, pg	0.776 ± 0.076	0.627 – 0.925	≤1.8	58.8%	91.3%
Ferritin, ng/mL	0.666 ± 0.089	0.491 – 0.841	≤145	70.6%	60.0%
TSAT, %	0.932 ± 0.036	0.861 – 1.000	≤19.8	94.1%	84.0%
Transferrin, mg/L	0.515 ± 0.096	0.328 – 0.703	≤250	58.8%	68.0%
Iron, µmol/L	0.922 ± 0.044	0.836 – 1.000	≤13	94.1%	88.0%
sTfR, mg/L	0.679 ± 0.089	0.505 – 0.852	≥1.06	70.6%	56.0%
sTfR-F index	0.706 ± 0.090	0.530 – 0.882	≥0.19	58.8%	92.0%
Hepcidin, nM	0.541 ± 0.111	0.322 – 0.761	≤6.1	52.9%	84.0%

ID=iron deficiency; RPI=reticulocyte production index; RDW=red blood cell distribution width; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration; HYPO=hypochromic red blood cells; RET-He=reticulocyte hemoglobin content; RBC-He=red blood cell hemoglobin content; Delta-He=difference between RBC-He and RET-He; TSAT=transferrin saturation; sTfR=soluble transferrin receptor; sTfR-F index=ratio between sTfR and log transformed ferritin.

bly, placing the cut-off at TSAT <20% provides the exact same results as TSAT ≤19.8%. Hemoglobin (AUC=0.820) and reticulocyte hemoglobin content (AUC=0.821) showed good diagnostic accuracy for ID as well. Diagnostic characteristics of the definition used in the clinical trials, TSAT ≤19.8% and serum iron ≤13µmol/L) are displayed in **Table 3**. The AUCs of TSAT (0.891) and serum iron (0.911) dichotomously analyzed were both significantly higher than the AUC of the definition used in the clinical trials (AUC=0.772; p=0.023 and p=0.046, respectively). Serum iron had a significant better specificity compared to the FAIR-HF definition (p=0.046), while there was a trend for a better specificity for TSAT (p=0.083). Sensitivities did not differ significantly. Furthermore, the addition of

ferritin to the ROC curve of TSAT or serum iron did not result in a significant increase in AUC (0.932 to 0.925; p=0.523 and 0.922 to 0.925; p=0.700). Other definitions based on hemoglobin, reticulocyte hemoglobin concentration, ferritin, sTfR, hepcidin, MCV and hypochromic red cells did not result in a significantly improved sensitivity or specificity compared to the FAIR-HF definition. The sTfR-ferritin index showed a higher specificity (92%, p=0.025), but has a low sensitivity (59%).

Table 3 – Diagnostic characteristics for ID of the FAIR-HF definition compared with TSAT and serum iron.

	FAIR-HF	TSAT ≤19.8%	Iron ≤13 µmol/L
Sensitivity, %	82.4	94.1	94.1
Specificity, %	72.0	84.0	88.0*
ROC-AUC	0.772	0.891*	0.911*
Likelihood Ratio (+)	2.94	5.88	7.84
Likelihood Ratio (-)	0.25	0.07	0.07
Odds Ratio	12.00	84.00	117.33
Positive Predictive Value, %	66.7	80.0	84.2
Negative Predictive Value, %	85.7	95.5	95.7

* P-value for difference with FAIR-HF criteria <0.05
ID=iron deficiency; TSAT=transferrin saturation; ROC=receiver operating characteristics; AUC=area under the curve.

TSAT, serum iron and prognosis

Since TSAT ≤19.8% and serum iron ≤13µmol/L were the optimal definitions of ID, and ID has been associated with impaired prognosis, we tested whether either of these simple definitions identifies patients at higher risk of all-cause mortality. Baseline characteristics are presented in **Supplemental Table 1**. Patients had a mean age of 67±13 years, 68% were male patients, the majority of patients were in NYHA class II or III (56% and 29%, respectively) and 48% had a history of myocardial infarction. Mean LVEF was 30±9% and median NT-proBNP was 1504 (interquartile range 656–3306). Of a total of 387 patients, 178 patients (48.0%) had a serum iron ≤13µmol/L, 165 (42.6%) had a TSAT≤19.8%, and 50 (12.9%) had a ferritin <100 ng/mL with a normal TSAT (>19.8%). 154 patients (39.8%) fulfilled the criteria for ID based on both serum iron and TSAT. Survival analyses (**Figure 2** and **Table 4**) showed that both a low TSAT and a low serum iron were significantly associated with the risk of death (hazard ratio 2.78, 95% confidence interval 1.22 – 6.34 and hazard ratio 2.39, 95% CI 1.13 – 5.04). In contrast, an isolated low ferritin was not significantly associated with the risk of death (hazard ratio 1.54, 95% CI 0.45 – 5.52).

Table 4 – Cox proportional hazard regression on all-cause mortality

	All-cause mortality		
	HR	**95% CI**	**P-value**
Univariable			
TSAT ≤19.8%	3.91	1.88 – 8.16	<0.001
Isolated low ferritin	1.28	0.39 – 4.15	0.686
Iron ≤13µmol/L	3.60	1.87 – 6.93	<0.001
Multivariable*			
TSAT ≤19.8%	2.78	1.22 – 6.34	0.015
Isolated low ferritin	1.54	0.45 – 5.22	0.488
Iron ≤13µmol/L	2.39	1.13 – 5.04	0.022

* Adjusted for age, sex, BMI, systolic blood pressure, New York Heart Association class, left ventricular ejection fraction, log transformed NT-proBNP, log transformed serum creatinine, sodium, hemoglobin, log transformed CRP, diabetes mellitus, COPD and beta-blocker and ACEi/ARB use. HR=hazard ratio; CI=confidence interval; HF=heart failure; TSAT=transferrin saturation; BMI=body mass index; COPD=chronic obstructive pulmonary disease; ACEi=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker.

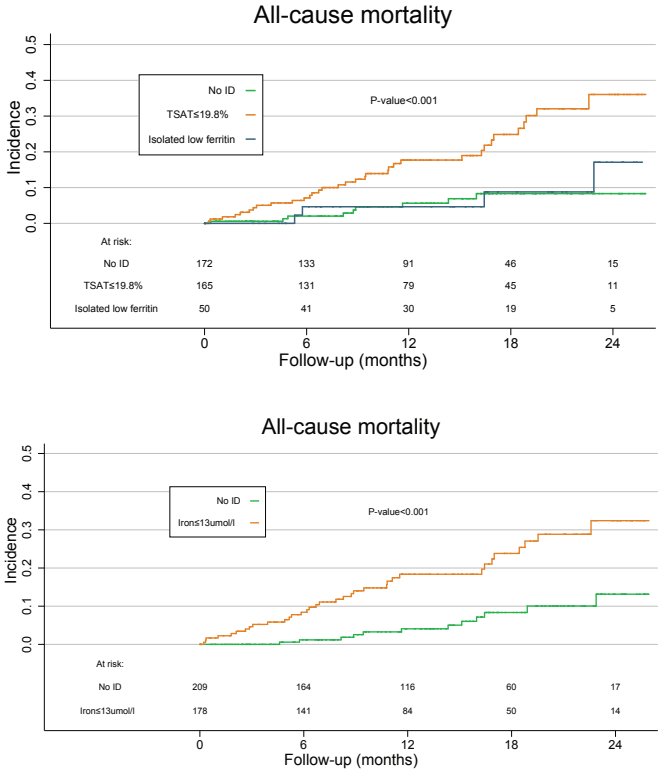

Figure 2 – TSAT ≤19.8% and iron≤13µmol/L and effect on all-cause mortality in the outpatient heart failure population. Kaplan-Meier of patients with either no iron deficiency, a low TSAT (≤19.8%) or an isolated low ferritin (<100 ng/mL), or no iron deficiency and a low serum iron (≤13µmol/L). The P-value for interaction between the three groups is noted. HF=heart failure; ID=iron deficiency; TSAT=transferrin saturation.

DISCUSSION

We herein show for the first time that for adequate assessment of ID, a TSAT ≤19.8% or a serum iron≤13µmol/L provide the best diagnostic accuracy for bone marrow ID, the gold standard of iron assessment. This confirms the currently used TSAT cut off of <20%. Supporting this, outpatient HF patients selected using this definition were at higher risk for all-cause death. Ferritin appears to be less useful in assessing ID. We validate the frequent occurrence of ID in HF, and identified ID in 40% of our patients with HF. Our data underscore the importance of ID as a co-morbidity in HF, but also indicate the importance of validating clinically used cut points of surrogate parameters.

In recent years, several studies assessed the prevalence of ID in HF patients using serum markers like ferritin, TSAT and hemoglobin, and prevalences around 50% are reported.[1] Only one study by Nanas et al. assessed iron status in patients with HF using the gold standard and found ID in a relatively high prevalence of 73% (27 of 37 patients included in the study).[25] This higher percentage compared to the present study can be explained by the inclusion of solely anemic patients with decompensated advanced HF.[25] Jankowska et al. assessed iron status using bone marrow aspiration in 65 patients with stable coronary artery disease. Absolute ID was present in 31 (48%) patients and serum soluble transferrin receptor, the erythropoietin receptor and TSAT were the most diagnostically accurate biomarkers, no data on serum iron was reported.[26] In our population of chronic HF patients, we report a prevalence of 40%, while anemia was present in only 17% of patients. The design of the current study, which included patients undergoing CABG surgery, might have caused a bias as patients with advanced HF rarely undergo CABG surgery. Therefore, the prevalence of ID might be even higher in an unselected HF population.

Large trials with intravenous FCM conducted in recent years such as the FAIR-HF, CONFIRM-HF (Ferric Carboxymaltose Evaluation on Performance in Patients With Iron Deficiency in Combination With Chronic Heart Failure), and EFFECT-HF (Effect of Ferric Carboxymaltose on Exercise Capacity in Patients With Iron Deficiency and Chronic Heart Failure), included patients with either low iron stores (ferritin <100 ng/mL) or low iron availability with normal stores (ferritin 100-300 ng/mL and TSAT <20%).[6,7,12] This definition of ID is also applied in most epidemiological studies, but the diagnostic accuracy in HF has never been tested. In our cohort, the criteria used in these studies had a sensitivity of 92.4% and specificity of 72.0%, indicating that 28.0% of patients with normal bone marrow iron would have been erroneously diagnosed with ID using those criteria. Additionally, we tested a large set of circulating hematological and iron parameters in comparison with the bone marrow staining results. The best indicators of ID are TSAT and serum iron. TSAT reflects the percentage of transferrin binding places

occupied with iron. We show that transferrin levels are not associated with bone marrow iron status in this cohort, consequently iron and TSAT values are closely linked to each other and show comparable findings. TSAT has an ROC calculated optimal cut-off value of 19.8%, very similar to the FAIR-HF criteria (<20%). The TSAT cut-off of 19.8% resulted in a sensitivity of 94.1% and specificity of 84.0%; at least as good as the FAIR-HF criteria. Further, a low ferritin (<100 ng/mL) was often accompanied by a low TSAT. However, the cases that presented with a low ferritin but a normal TSAT were not diagnosed with ID. Addition of ferritin to the ROC model did also not result in an increase in diagnostic accuracy.

To be able to apply either of these definitions of ID, using TSAT or serum iron, in clinical practice and to determine the treatment options for the individual patient, it is essential to know if patients selected using these definitions have worse prognosis compared to patients without ID and if they benefit from treatment in terms of outcome. To assess prognostic consequences, we used an outpatient HF cohort including patients from the same center as the bone marrow study. We excluded patients with a preserved LVEF or receiving iron at baseline, either intravenously or orally. Small differences consisted in a lower mean LVEF and higher median NT-proBNP level in the outpatient HF cohort. We found that patients with ID according to the optimal definition using either TSAT or serum iron are at higher risk of death. Patients with isolated low ferritin levels, which did not correlate with bone marrow ID, had similar prognosis to those with normal ferritin and TSAT levels. Comparable results were found by Moliner et al. in an international cohort of 1821 patients with HF.[27] In that study, patients were divided into 3 groups of impaired iron status, either isolated low ferritin (<100 ng/mL) or TSAT (<20%) or a combination of both. They report 12% of patients having isolated low ferritin levels and 46% with low TSAT levels, independently of ferritin. An impaired TSAT level was associated with higher NT-proBNP levels, worse quality of life and higher incidence of all-cause mortality compared to isolated impaired ferritin levels.

Only the IRON-HF trial assessed treatment benefit in a population of ID diagnosed using a similar cutoff: TSAT <20%, with a ferritin <500 ng/mL.[28] Increased VO_2max levels were found in patients treated with intravenous iron. However, the study was underpowered due to premature termination and no statistically significant differences were found. The first trial with intravenous iron therapy by Toblli et al. was performed in 40 anemic HF patients with either a TSAT <20% or a ferritin <100 ng/mL and reported significant improvement in NT-proBNP and inflammatory status and better functional outcome regarding, among other factors, LVEF and NYHA class.[29] Unfortunately, no subgroup data based on TSAT <20% independently of ferritin levels or serum iron were reported. However, a recent meta-analysis did assess subgroups.[8] This meta-analysis used individ-

ual patient data of the four randomized controlled trials comparing FCM with placebo in patients with systolic HF and ID according to the FAIR-HF definition.[8] The primary endpoint studied was recurrent cardiovascular hospitalizations and cardiovascular mortality. Overall, FCM had a beneficial effect on prognosis. More interestingly, considering the results of our study, was the subgroup analysis showing an interaction with tertiles of TSAT levels on prognosis. Patients in the lower TSAT tertiles had significantly more benefit from treatment with FCM. No interactions were found for hemoglobin or ferritin levels. With support of the sponsor of the four studies, we applied the cutoff of a TSAT ≤19.8% and serum iron ≤13µmol/L to the primary endpoint of the previously published meta-analysis. The results are depicted in **Figure 3**. Strictly hypothesis generating, these results suggest that patients with a TSAT ≤19.8% had a significantly improved outcome with regard to cardiovascular hospitalization and cardiovascular death after treatment with FCM while patients with low ferritin, but a TSAT >19.8% did not benefit from FCM treatment. Similarly, patients with a serum iron ≤13µmol/L showed improved outcome after treatment while those with a serum iron >13µmol/L did not.

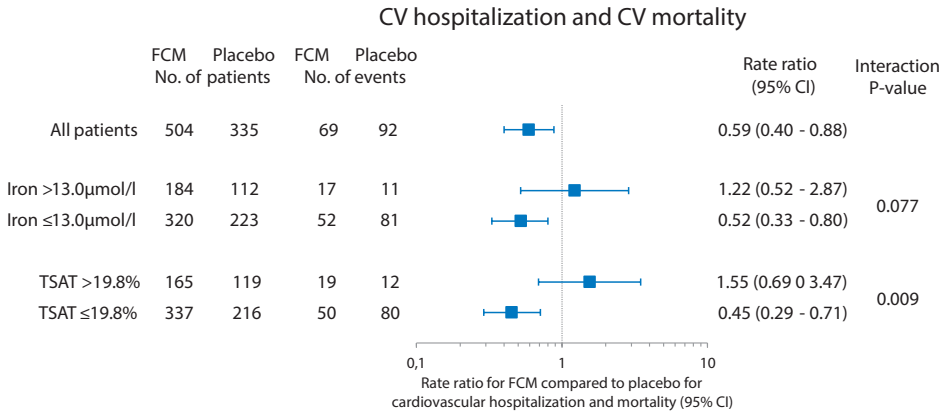

Figure 3 – TSAT ≤19.8% and iron≤13µmol/L and effect on cardiovascular hospitalizations and cardiovascular mortality in the randomized placebo controlled clinical trials with ferric carboxymaltose. Subgroup analysis of patients with either a low or normal TSAT or serum iron for outcome data of the trials with FCM. FCM=ferric carboxymaltose; TSAT=transferrin saturation.

These findings, in addition to the bone marrow results and data on prognosis, further support the hypothesis that patients with an isolated low ferritin (without a low TSAT or low serum iron) do not have ID and that this group of patients might receive intravenous iron without clear benefit: see **Figure 4** for a complete overview of the presented data. This could be of importance for clinical practice and suggests that future trials might be designed with this in mind. Importantly, the meta-analysis did not include patients with a ferritin >300 ng/mL. Although our bone marrow results indicate that those patients with a low TSAT and high ferritin are iron deficient, we cannot make statements regard-

ing treatment effect in this specific patient group. One might postulate that ferritin should not be used to diagnose ID in patients with HF, but perhaps can be used as a safety parameter to avoid iron treatment in patients with potential iron overload.[30] It has to be noted that the sensitivity of ferritin to diagnose iron overload is high, but the specificity is low due to many other conditions that can lead to high levels.[30]

We are not the first to propose the use of serum iron or TSAT to assess ID, without taking ferritin into account. Okonko et al. studied iron status based on circulating markers, in 157 HF patients and found that low TSAT and mean corpuscular hemoglobin concentration concentrations were present in 43% of patients despite often normal ferritin levels.[3] It was concluded that iron handling is abnormal in HF and that iron is directed from the circulation and erythroid marrow to the storage sites, making ferritin less reliable as a marker of iron accessible for the erythron and other tissues.

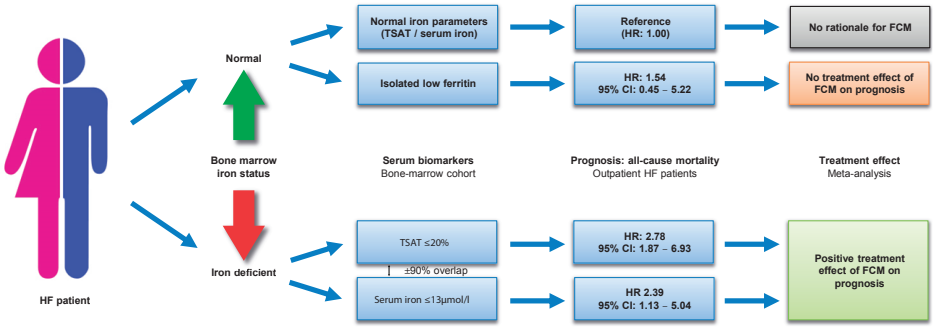

Figure 4 – Central Illustration – The validation of serum biomarkers for the diagnosis of iron deficiency. Bone marrow iron status, considered the gold standard, was assessed in 42 patients with heart failure. Using receiver operator characteristic analysis, transferrin saturation and serum iron were identified as the best biomarkers for iron status. Subsequently, the association of iron deficiency using the optimal biomarkers with mortality was shown in 387 outpatient heart failure patients. In an individual patient data (n=839) meta-analysis of randomized controlled trials with intravenous ferric carboxymaltose, those patients fulfilling the obtained criteria for iron deficiency responded to treatment with improved prognosis, while those not fulfilling the criteria did not. HF=heart failure; TSAT=transferrin saturation; HR=hazard ratio; CI=confidence interval; FCM=ferric carboxymaltose.

Strengths and limitations

A strength of this study is that we assessed iron status using the gold standard, taking both iron stores and iron availability into account in order to be able to include both absolute and functional shortage of iron as ID. Furthermore, we applied a large panel of biomarkers to find the best predictor of ID and subsequently tested the prognostic performance of this biomarker in an outpatient environment. The design of the study is a limitation as the bone marrow study is a relatively small single-center study and we

only investigated HF patients with coronary artery disease scheduled for CABG. Consequently, our findings may not be applicable to other HF populations. Although several authors report a circadian rhythm for iron, and therefore also TSAT, levels were found to be relatively stable during daytime.[31,32]

CONCLUSION

ID, assessed using the gold standard of bone marrow staining, is common in patients with HF. A TSAT ≤19.8% or a serum iron ≤13μmol/L show the best performance in selecting patients with ID and identifies HF patients at the highest risk of death. Our findings validate the currently used TSAT cut-off of <20% for the identification of ID in HF patients, and call into question the value of serum ferritin in the assessment of ID.

DISCLOSURES

NGB received personal fees from Vifor Pharma. ITK received speaker fees from Vifor Pharma. AAV received consultancy fees and an unrestricted grant from Vifor Pharma. WCM, ELV, HHW, DS, JvP, ABM, SKB, EV and MAM declare no competing interest. RadB received research funding from Bristol Meyers Squibb, AstraZeneca, and Trevena, outside the work for the current study. DJvV received Board Membership Fees from Vifor Pharma. PvdM received consultancy fees and an unrestricted grant from Vifor Pharma.

REFERENCES

1. Klip IT, Comin-Colet J, Voors AA, Ponikowski P, Enjuanes C, Banasiak W, Lok DJ, Rosentryt P, Torrens A, Polonski L, van Veldhuisen DJ, van der Meer P, Jankowska EA. Iron deficiency in chronic heart failure: an international pooled analysis. *Am Heart J*. 2013;165:575–582. e3.
2. Jankowska EA, Rozentryt P, Witkowska A, Nowak J, Hartmann O, Ponikowska B, Borodulin-Nadzieja L, Banasiak W, Polonski L, Filippatos G, McMurray JJ V, Anker SD, Ponikowski P. Iron deficiency: An ominous sign in patients with systolic chronic heart failure. *Eur Heart J*. 2010;31:1872–1880.
3. Okonko DO, Mandal AKJ, Missouris CG, Poole-Wilson PA. Disordered iron homeostasis in chronic heart failure: Prevalence, predictors, and relation to anemia, exercise capacity, and survival. *J Am Coll Cardiol*. 2011;58:1241–1251.
4. van Veldhuisen DJ, Anker SD, Ponikowski P, Macdougall IC. Anemia and iron deficiency in heart failure: mechanisms and therapeutic approaches. *Nat Rev Cardiol*. 2011;8:485–93.
5. Harris S, Tepper D, Ip R. Effect of intravenous iron sucrose on exercise tolerance in anemic and nonanemic patients with symptomatic chronic heart failure and iron deficiency-ferric-hf: A randomized, controlled, observer-blinded trial. *Congest Hear Fail*. 2009;15:208.
6. Anker SD, Comin Colet J, Filippatos G, Willenheimer R, Dickstein K, Drexler H, Lüscher TF, Bart B, Banasiak W, Niegowska J, Kirwan B, Mori C, von Eisenhart Rothe B, Pocock SJ, Poole-Wilson PA, Ponikowski P. Ferric Carboxymaltose in Patients with Heart Failure and Iron Deficiency. *N Engl J Med*. 2009;361:2436–2448.
7. Ponikowski P, Van Veldhuisen DJ, Comin-Colet J, Ertl G, Komajda M, Mareev V, McDonagh T, Parkhomenko A, Tavazzi L, Levesque V, Mori C, Roubert B, Filippatos G, Ruschitzka F, Anker SD. Beneficial effects of long-term intravenous iron therapy with ferric carboxymaltose in patients with symptomatic heart failure and iron deficiency. *Eur Heart J*. 2015;36:657–668.
8. Anker SD, Kirwan B-A, van Veldhuisen DJ, Filippatos G, Comin-Colet J, Ruschitzka F, Lüscher TF, Arutyunov GP, Motro M, Mori C, Roubert B, Pocock SJ, Ponikowski P. Effects of ferric carboxymaltose on hospitalisations and mortality rates in iron-deficient heart failure patients: an individual patient data meta-analysis. *Eur J Heart Fail*. 2018;20:125–133.
9. Weiss G, Goodnough LT. Anemia of Chronic Disease. *Inflammation*. 2012;352:1011–1023.
10. Lok DJ, Klip IT, Lok SI, de La Porte A, Badings E, Van Wijngaarden J, Voors AA, De Boer RA, Van Veldhuisen DJ, Van Der Meer P. Incremental prognostic power of novel biomarkers (growth-differentiation factor-15, high-sensitivity C-reactive protein, galectin-3, and high-sensitivity troponin-T) in patients with advanced chronic heart failure. *Am J Cardiol*. 2013;112:831–837.
11. Garcia-Casal MN, Peña-Rosas JP, Pasricha S-R. Rethinking ferritin cutoffs for iron deficiency and overload. *Lancet Haematol*. 2014;1:e92-4.
12. van Veldhuisen DJ, Ponikowski P, van der Meer P, Metra M, Böhm M, Doletsky A, Voors AA, Macdougall IC, Anker SD, Roubert B, Zakin L, Cohen-Solal A. Effect

of Ferric Carboxymaltose on Exercise Capacity in Patients With Chronic Heart Failure and Iron Deficiency. *Circulation*. 2017;136:1374–1383.

13. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2016;37:2129–2200m.
14. Baumgartner-Stäubli R, Beck EA. Sideroblast Score: A Sensitive Indicator of Iron Deficiency and Hypoproliferative Anemia. *Acta Haematol*. 2009;57:24–31.
15. Hansen HA, Weinfeld A. Hemosiderin Estimations and Sideroblast Counts in the Differential Diagnosis of Iron Deficiency and other Anemias. *Acta Med Scand*. 1959;165:333–356.
16. Gale E, Torrance J, Bothwell T. The quantitative estimation of total iron stores in human bone marrow. *J Clin Invest*. 1963;42:1076–1082.
17. Phiri KS, Calis JCJ, Kachala D, Borgstein E, Waluza J, Bates I, Brabin B, van Hensbroek MB. Improved method for assessing iron stores in the bone marrow. *J Clin Pathol*. 2009;62:685–9.
18. Kroot JJC, Laarakkers CMM, Geurts-Moespot AJ, Grebenchtchikov N, Pickkers P, Van Ede AE, Peters HPE, Van Dongen-Lases E, Wetzels JFM, Sweep FCGJ, Tjalsma H, Swinkels DW. Immunochemical and mass-spectrometry-based serum hepcidin assays for iron metabolism disorders. *Clin Chem*. 2010;56:1570–1579.
19. Beilby J, Olynyk J, Ching S, Prins A, Swanson N, Reed W, Harley H, Garcia-Webb P. Transferrin index: An alternative method for calculating the iron saturation of transferrin. *Clin Chem*. 1992;38:2078–2081.
20. WHO scientific group. Nutritional anaemias. Report of a WHO group of experts. *World Heal Organ - Tech Rep Ser*. 1972;503:1–29.
21. Punnonen K, Irjala K, Rajamäki a. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood*. 1997;89:1052–1057.
22. Matsushita K, Mahmoodi BK, Woodward M, Emberson JR, Jafar TH, Jee SH, Polkinghorne KR, Shankar A, Smith DH, Tonelli M, Warnock DG, Wen C-P, Coresh J, Gansevoort RT, Hemmelgarn BR, Levey AS. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. *Jama*. 2012;307:1941–51.
23. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametetric approach. *Biometrics*. 1988;44:837–845.
24. Pocock SJ, Ariti CA, McMurray JJ V, Maggioni A, Køber L, Squire IB, Swedberg K, Dobson J, Poppe KK, Whalley GA, Doughty RN, Meta-Analysis Global Group in Chronic Heart Failure. Predicting survival in heart failure: a risk score based on 39 372 patients from 30 studies. *Eur Heart J*. 2013;34:1404–13.
25. Nanas JN, Matsouka C, Karageorgopoulos D, Leonti A, Tsolakis E, Drakos SG, Tsagalou EP, Maroulidis GD, Alexopoulos GP, Kanakakis JE, Anastasiou-Nana MI. Etiology of Anemia in Patients With Advanced Heart Failure. *J Am Coll Cardiol*. 2006;48:2485–2489.
26. Jankowska EA, Wojtas K, Kasztura M, Mazur G, Butrym A, Kalicinska E, Rybinska I, Skiba J, Von Haehling S, Doehner W, Anker SD, Banasiak W, Cleland

JGF, Ponikowski P. Bone marrow iron depletion is common in patients with coronary artery disease. *Int J Cardiol*. 2015;182:517–522.

27. Moliner P, Jankowska EA, van Veldhuisen DJ, Farre N, Rozentryt P, Enjuanes C, Polonski L, Meroño O, Voors AA, Ponikowski P, Van der Meer P, Comin-Colet J. Clinical correlates and prognostic impact of impaired iron storage versus impaired iron transport in an international cohort of 1821 patients with chronic heart failure. *Int J Cardiol*. 2017;243:360–366.

28. Beck-Da-Silva L, Piardi D, Soder S, Rohde LE, Pereira-Barretto AC, De Albuquerque D, Bocchi E, Vilas-Boas F, Moura LZ, Montera MW, Rassi S, Clausell N. IRON-HF study: A randomized trial to assess the effects of iron in heart failure patients with anemia. *Int J Cardiol*. 2013;168:3439–3442.

29. Toblli JE, Lombraña A, Duarte P, Di Gennaro F. Intravenous Iron Reduces NT-Pro-Brain Natriuretic Peptide in Anemic Patients With Chronic Heart Failure and Renal Insufficiency. *J Am Coll Cardiol*. 2007;50:1657–1665.

30. Wood JC. Guidelines for quantifying iron overload. *Hematol Am Soc Hematol Educ Progr*. 2014;2014:210–215.

31. Dale JC, Burritt MF, Zinsmeister AR. Diurnal variation of serum iron, iron-binding capacity, transferrin saturation, and ferritin levels. *Am J Clin Pathol*. 2002;117:802–808.

32. Ridefelt P, Larsson A, Rehman J ur, Axelsson J. Influences of sleep and the circadian rhythm on iron-status indices. *Clin Biochem*. 2010;43:1323–1328.

Supplemental Material

Supplemental Table 1 – Baseline characteristics of the outpatient HF patients.

Variable	Total	No ID	TSAT ≤19.8%	Isolated low ferritin	P-value*
N	387	172	165	50	
Age, y	66.8 ± 13.4	66.1 ± 13.5	66.7 ± 13.6	69.0 ± 12.4	0.40
Female gender	124 (32.0%)	44 (25.6%)	59 (35.8%)	21 (42.0%)	0.037
BMI, kg/m2	27.7 ± 5.3	27.8 ± 5.3	27.8 ± 5.4	27.4 ± 5.0	0.89
SBP (mmHg)	119.2 ± 21.3	115.7 ± 18.0	121.4 ± 22.9	124.1 ± 24.6	0.012
NYHA class					0.017
1	48 (12.4%)	23 (13.4%)	16 (9.7%)	9 (18.0%)	
2	216 (55.8%)	107 (62.2%)	81 (49.1%)	28 (56.0%)	
3	112 (28.9%)	36 (20.9%)	63 (38.2%)	13 (26.0%)	
4	11 (2.8%)	6 (3.5%)	5 (3.0%)	0 (0.0%)	
LVEF, %	30.4 ± 9.3	30.6 ± 9.2	29.9 ± 9.3	31.6 ± 9.3	0.50
Comorbidities					
Previous MI	187 (48.3%)	74 (43.0%)	86 (52.1%)	27 (54.0%)	0.17
Diabetes mellitus	133 (34.4%)	53 (30.8%)	64 (38.8%)	16 (32.0%)	0.28
Atrial fibrillation	173 (44.7%)	82 (47.7%)	76 (46.1%)	15 (30.0%)	0.078
Hypertension	165 (42.6%)	67 (39.0%)	75 (45.5%)	23 (46.0%)	0.42
Hypercholesterolemia	297 (76.7%)	135 (78.5%)	120 (72.7%)	42 (84.0%)	0.20
ID (FAIR-HF)	199 (51.4%)	6 (3.5%)	143 (86.7%)	50 (100.0%)	<0.001
Anemia	99 (25.6%)	20 (11.6%)	72 (43.6%)	7 (14.0%)	<0.001
Laboratory values					
NT-proBNP, ng/L	1504 (656, 3306)	1180 (522, 2649)	2078 (803, 4749)	1379 (651, 2578)	<0.001
eGFR, mL/min/1.73m2	62.4 ± 26.3	63.9 ± 24.6	60.9 ± 29.1	61.7 ± 21.9	0.58
Sodium, mmol/L	139.9 ± 3.2	140.2 ± 2.9	139.4 ± 3.5	140.6 ± 3.3	0.025
LDH, U/L	219 (185, 255)	217 (183, 252)	227 (189, 260)	206 (187, 268)	0.36
CRP, mg/L	3.8 (1.6, 8.6)	2.8 (1.2, 7.2)	5.7 (2.8, 12.0)	2.5 (1.3, 3.8)	<0.001
HbA1c, %	6.1 (5.8 – 6.7)	6.1 (5.8 – 6.6)	6.2 (5.8 – 6.7)	6.1 (5.8 – 6.6)	0.49
HDL/LDL ratio	0.45 (0.33 – 0.62)	0.42 (0.32 – 0.57)	0.47 (0.34 – 0.67)	0.52 (0.36 – 0.64)	0.13
AST, U/L	26.0 (20.0, 33.0)	28 (23, 35)	25 (20, 30)	24 (20, 32)	<0.001
ALT, U/L	22.0 (15.0, 31.0)	24.0 (16.0, 37.0)	19.0 (14.0, 26.0)	20 (14, 28)	<0.001
Hematology					
Hemoglobin, g/dL	13.8 ± 1.9	14.6 ± 1.7	12.8 ± 1.8	14.2 ± 1.3	<0.001
Hematocrit, %	0.42 ± 0.05	0.44 ± 0.05	0.40 ± 0.05	0.43 ± 0.04	<0.001
Iron, µmol/L	14 (10 – 19)	18 (16 – 22)	10 (7 – 12)	17 (15 – 22)	<0.001

Supplemental Table 1 – Baseline characteristics of the outpatient HF patients. (*continued*)

Variable	Total	No ID	TSAT ≤19.8%	Isolated low ferritin	P-value*
Ferritin, ng/mL	147 (70, 282)	249 (166, 394)	85 (44, 175)	75 (52, 86)	<0.001
TSAT, %	21.9 (15.1, 28.9)	28.7 (24.6, 34.3)	13.7 (10.6, 17.0)	24.6 (22.3, 29.8)	<0.001
Transferrin, mg/dL	268.4 ± 45.4	251.6 ± 35.8	283.7 ± 50.8	275.8 ± 35.7	<0.001
Medication					
Anti-platelet therapy	63 (16.3%)	25 (14.5%)	25 (15.2%)	13 (26.0%)	0.14
Diuretics	296 (76.5%)	133 (77.3%)	129 (78.2%)	34 (68.0%)	0.31
β-blocker	353 (91.2%)	164 (95.3%)	144 (87.3%)	45 (90.0%)	0.031
ACEi or ARB	326 (84.2%)	148 (86.0%)	136 (82.4%)	42 (84.0%)	0.66
MRA	190 (49.1%)	85 (49.4%)	85 (51.5%)	20 (40.0%)	0.36
OAC	224 (57.9%)	99 (57.6%)	102 (61.8%)	23 (46.0%)	0.14

* Normal vs. patients with TSAT ≤19.8% and patients with an isolated low ferritin.
Data are presented as mean ± standard deviation when normally distributed, as median and interquartile range when non-normally distributed, or as frequencies and percentages for categorical variables.
BMI=body mass index; SBP=systolic blood pressure; NYHA class=New York Heart Association class; LVEF=left ventricular ejection fraction; MI=myocardial infarction; ID=iron deficiency; eGFR=estimated glomerular filtration rate; LDH=lactate dehydrogenase; CRP=c-reactive protein; HDL=high density lipoprotein; LDL=low density lipoprotein; AST=aspartate transferase; ALT=alanine transferase; TSAT=transferrin saturation; ACEi=angiotensin converting enzyme inhibitor; ARB=angiotensin receptor blocker; MRA=mineralocorticoid receptor antagonists, OAC=oral anticoagulants.