Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods.

Bone marrow cohort

Patients scheduled for coronary artery bypass graft (CABG) surgery at the University Medical Center Groningen, Groningen, The Netherlands were eligible. Inclusion criteria were a history of HF with a reduced left ventricular ejection fraction (LVEF ≤45%) and a plasma NT-proBNP concentration of >125 ng/L. The major exclusion criteria were a history of acquired iron overload, iron therapy in the previous year or any disease known to influence iron metabolism, such as severe renal failure (estimated glomerular filtration rate [eGFR] <30 ml/min/1,73m^2), infection or hematological diseases. A total of 50 patients were included in the study but data were incomplete in 8 cases (6 failed bone marrow assessments because of insufficient material and 2 patients did not undergo surgery).

BIOSTAT-CHF cohort

The differential impact of LIS and DIU on clinical and biochemical parameters and prognosis was evaluated in a pan-European cohort of patients with worsening HF (A systems BIOlogy Study to Tailored Treatment in Chronic Heart Failure; BIOSTAT-CHF). In brief, this study enrolled subjects with worsening HF who either presented at the outpatient clinic or were hospitalized for worsening HF. Main inclusion criteria were LVEF ≤40% or NT-proBNP/BNP levels of >2000 ng/L or >400 ng/L, respectively. Moreover, subjects needed to be treated sub-optimally according to heart failure treatment guidelines (i.e. ≤50% of target dose of beta-blockers and/or angiotensin-converting enzyme inhibitors or angiotensin receptor blockers). More details on the BIOSTAT-CHF study have been published previously.[16–19] Subjects were prospectively followed for a median follow-up of 21 months (IQR, 16–27 months).

Analytical methods

Bone marrow aspirates were taken from the sternum during CABG. Multiple Prussian blue stained slides per sample were assessed by two independent analysts in a certified core-lab for the presence of non-heme bound iron. Functional availability of iron for erythropoiesis was assessed by the percentage of erythroblasts containing iron, i.e. sideroblasts.[12] In normal conditions, 20-50% of the erythroblasts contain iron, 10-20% is considered low normal, and <10% is considered iron deficient.[13] To distinguish LIS from DIU, the amount of iron present in the extracellular space (storage) was graded using Gale's method.[14] 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". Patients with sideroblasts <10% and depleted iron stores were classified as LIS, while patients with sideroblasts <10% and normal iron stores were classified as DIU.[15]

An extensive hematological profile was analyzed in fresh venous blood with ethylenediaminetetraacetic acid using the Sysmex XN20 (Sysmex Corporation, Kobe, Japan). Markers of iron status were assessed using standard methods on a Roche Modular Cobas 8000 (Roche Diagnostics, Indianapolis, USA). 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.[1] 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.[2] All laboratory measurements were done in fresh venous blood except for serum soluble transferrin receptor and hepcidin

and the hematologic and iron parameters measured in the BIOSTAT-CHF cohort. These were measured in serum stored at -80°C 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.[3] 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.[4] 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.[5] 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. Left sided congestion was defined as pulmonary congestion on auscultation or orthopnea. Rights sided congestion was defined as the presence of edema above the ankles, an elevated venous jugular pressure or hepatomegaly. Daily protein intake was estimated from spot urine samples using the adjusted Maroni formula, as

previously described in chronic kidney disease: 13.9 + 0.907 * body mass index (BMI) (kg/m2) + 0.0305 * urinary urea nitrogen level (mg/dL).[6]

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 students t-test, Wilcoxon 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 separate LIS (scored as "1") from DIU (scored as "0"). The area under the curve (AUC) reflects the performance of the test with a score >0.80 considered as a good accuracy and >0.70 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: $d^2 = (1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$.

In the BIOSTAT-CHF cohort, univariable and multivariable logistic regression analyses were performed with iron status as independent and clinical variables as dependent variable. All variables previously included in the BIOSTAT-CHF prediction model were used in the multivariable analyses with the exception of hemoglobin, edema, eGFR and blood urea nitrogen as these were included as the dependent variables, the variables included are: age, previous heart failure hospitalization, systolic blood pressure, natural log transformed NT-proBNP, HDL, sodium and beta-blocker use.[7] Dependent continuous variables were dichotomized based on either clinical useful cut-offs (LVEF <30%, eGFR <60mL/min/1.73m^2) or the median (6-minute walking test distance and Kansas City Cardiomyopathy Questionnaire overall

summary score). Cox proportional hazard regression analyses were performed univariable and in a multivariable model including all variables included in the BIOSTAT-CHF prediction model and additionally corrected for hemoglobin.[7] The BIOSTAT-CHF model for the composite endpoint consisted of: age, previous heart failure hospitalization, edema, systolic blood pressure, natural log transformed NT-proBNP, hemoglobin, HDL, sodium and beta-blocker use. HF-hospitalizations were assessed with the following covariates: age, previous heart failure hospitalization, edema, systolic blood pressure and estimated glomerular filtration rate. Finally, the model for all-cause mortality consisted of: age, natural log transformed blood urea nitrogen, natural log transformed NT-proBNP, hemoglobin and beta-blocker use. Fractional polynomial hazard regression analyses were performed to assess the best-fitting functional form for iron parameters and its association with prognosis. Follow-up was truncated when <5% of the subjects were at risk, which was at 2.8 years. Missing values for all BIOSTAT-CHF prediction model variables were imputed as described by Voors et al., after which all prediction models were averaged.[7]

We considered a two-sided P-value of <0.05, or <0.10 for interaction analyses, statistically significant. All tests and analyses were performed using STATA version 15.0 (StataCorp LP, College Station, Texas, USA) and GraphPad Prism version 5.04 (GraphPad Software Inc., La Jolla, USA).

eTable 1. Baseline characteristics of the bone marrow cohort

Variable	Total	No ID	DIU	LIS	P-value*
N	42	25	8	9	
Age, y	68.0 ± 9.5	67.4 ± 9.6	66.5 ± 11.8	70.8 ± 7.6	0.38
Female gender	10 (24%)	5 (20%)	1 (13%)	4 (44%)	0.15
BMI, kg/m2	28.6 ± 3.8	28.6 ± 3.4	28.4 ± 5.2	29.0 ± 4.2	0.78
SBP (mmHg)	131.5 ± 16.5	132.2 ± 14.8	133.8 ± 21.8	127.4 ± 17.4	0.52
NYHA class					0.96
1	8 (19%)	6 (24%)	1 (13%)	1 (11%)	
2	21 (50%)	13 (52%)	4 (50%)	4 (44%)	
3	12 (29%)	5 (20%)	3 (38%)	4 (44%)	
4	1 (2%)	1 (4%)	0 (0%)	0 (0%)	
LVEF, %	37.8 ± 7.0	38.9 ± 7.4	35.2 ± 6.3	37.2 ± 6.6	0.54
Comorbidities					
Previous MI	20 (48%)	9 (36%)	4 (50%)	7 (78%)	0.23
Diabetes mellitus	22 (52%)	10 (40%)	4 (50%)	8 (89%)	0.079
Atrial fibrillation	12 (29%)	10 (40%)	0 (0%)	2 (22%)	0.16
Hypertension	32 (76%)	20 (80%)	7 (88%)	5 (56%)	0.15
Hypercholesterolemia	39 (93%)	24 (96%)	7 (88%)	8 (89%)	0.93
Anemia	7 (17%)	2 (8%)	1 (13%)	4 (44%)	0.15
Laboratory values					
NT-proBNP, ng/l	914 (454. 1755)	718 (436. 1749)	1136 (772. 1583)	1754 (324. 2555)	0.77
eGFR, ml/min/1,73m2	77.9 ± 18.8	78.8 ± 15.4	85.8 ± 19.1	68.5 ± 24.8	0.13
Sodium, mmol/l	139.8 ± 3.0	140.0 ± 3.1	139.0 ± 3.1	139.8 ± 3.2	0.62
LDH, U/l	175 (163. 191)	174 (163. 188)	179 (144. 198)	180 (167. 205)	0.67
CRP, mg/l	2.0 (0.9. 4.5)	1.5 (0.7. 2.1)	12.4 (1.2. 20.5)	2.7 (1.8. 3.5)	0.31
ESR, mm/hour	14 (4. 32)	8 (3. 18)	35 (8. 54)	32 (21. 35)	0.70
HbA1c, %	6.3 (5.7. 7.0)	5.8 (5.6. 6.6)	6.2 (6.0. 6.4)	7.3 (6.8. 7.5)	0.030
HDL/LDL ratio	0.48 (0.36. 0.62)	0.48 (0.36. 0.59)	0.51 (0.26. 0.92)	0.42 (0.40. 0.62)	1.00

AST, U/l	22 (19. 27)	24 (20. 27)	20 (18. 23)	23 (19. 24)	0.24
ALT, U/l	20 (16. 24)	20 (17. 26)	20 (12. 21)	16 (15. 20)	0.70
Hematology					
Hemoglobin, g/dl	14.0 ± 1.3	14.6 ± 1.1	13.7 ± 0.8	12.6 ± 1.0	0.020
Hematocrit, %	0.42 ± 0.03	0.43 ± 0.03	0.41 ± 0.02	0.39 ± 0.03	0.23
Reticulocytes, ‰	13.2 ± 4.3	12.6 ± 4.1	12.0 ± 4.8	16.0 ± 3.8	0.077
RPI	56.4 ± 18.3	59.4 ± 18.7	49.4 ± 18.7	54.5 ± 16.8	0.56
RDW, %	13.7 ± 1.8	13.1 ± 0.9	13.9 ± 2.7	15.1 ± 2.0	0.30
MCV, fl.	90.1 ± 5.3	91.1 ± 5.1	89.8 ± 4.9	87.6 ± 5.8	0.41
MCH, fmol	1881 ± 151	1931 ± 127	1866 ± 124	1759 ± 169	0.18
MCHC, g/dl	20.87 ± 0.82	21.19 ± 0.58	20.74 ± 0.86	20.11 ± 0.88	0.17
Ferritin, ng/ml	144 (85. 263)	159 (107. 271)	212 (144. 311)	44 (27. 70)	0.001
TSAT, %	20.9 (14.7. 27.8)	27.5 (21.3. 31.8)	17.9 (12.1. 19.8)	14.0 (7.9. 14.7)	0.067
HYPO, %	0.1 (0.1. 0.2)	0.1 (0.1. 0.1)	0.1 (0.0. 0.2)	0.4 (0.2. 0.6)	0.024
RET-He, pg	32.09 ± 2.56	33.21 ± 1.59	31.68 ± 1.81	29.58 ± 3.36	0.14
RBC-He, pg	29.86 ± 2.34	30.64 ± 1.74	29.79 ± 2.22	27.90 ± 2.81	0.15
Delta-He, pg	2.23 ± 0.83	2.56 ± 0.74	1.89 ± 0.72	1.67 ± 0.80	0.58
sTfR, mg/l	1.09 (0.94. 1.42)	1.05 (0.92. 1.24)	1.04 (0.93. 1.14)	1.59 (1.16. 1.88)	0.027
sTfR-F index	0.15 (0.13. 0.19)	0.15 (0.13. 0.17)	0.14 (0.12. 0.15)	0.34 (0.24. 0.36)	0.001
Hepcidin, nM	10.8 (5.9. 15.8)	11.4 (7.1. 13.9)	28.4 (12.6. 35.7)	1.2 (0.4. 5.9)	0.001
Medication					
Diuretics	22 (52%)	14 (56%)	2 (25%)	6 (67%)	0.086
β-blocker	32 (76%)	21 (84%)	5 (63%)	6 (67%)	0.86
ACEi/ARB	38 (90%)	23 (92%)	7 (88%)	8 (89%)	0.93
Aldosteron antagonist	12 (29%)	5 (20%)	3 (38%)	4 (44%)	0.77
Anti-platelet therapy	33 (79%)	17 (68%)	8 (100%)	8 (89%)	0.33
OAC	10 (24%)	8 (32%)	0 (0%)	2 (22%)	0.16

Legend. * LIS vs. DIU 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; OAC=oral anticoagulants.

eTable 2. Receiver operating characteristics for LIS vs DIU in patients with a TSAT <20%

Variables for distinction LIS/DIU*	AUC ± SE	± 95% CI	ROC defined optimal cut-off value
Hemoglobin, g/dl	0.79 ± 0.12	0.55 – 1.00	≤13.5
Hematocrit, %	0.64 ± 0.15	0.36 – 0.93	≤0.40
Reticulocytes, ‰	0.73 ± 0.14	0.46 – 1.00	≥16
RPI	0.54 ± 0.17	0.20 – 0.88	≥41.3
MCV, fL	0.56 ± 0.15	0.26 – 0.87	≤88.6
MCH, fmol	0.76 ± 0.15	0.47 – 1.0	≤1873
MCHC, g/dl	0.68 ± 0.15	0.38 – 0.97	≤20.1
RDW, %	0.80 ± 0.14	0.52 – 1.00	≥13.5
HYPO, %	0.79 ± 0.12	0.56 – 1.00	≥0.2
RET-He, pg	0.76 ± 0.14	0.49 – 1.00	≤32.1
RBC-He, pg	0.74 ± 0.14	0.46 – 1.00	≤29.8
Delta-He, pg	0.55 ± 0.15	0.24 – 0.85	≤1.2
Ferritin, ng/mL	0.97 ± 0.04	0.89 – 1.00	≤128
TSAT, %	0.73 ± 0.15	0.45 – 1.00	≤16.3
sTfR, mg/l	0.81 ± 0.12	0.57 – 1.00	≥1.1
sTfR/log ferritin index	0.97 ± 0.04	0.89 – 1.00	≥0.19
Hepcidin, nM	0.97 ± 0.04	0.89 – 1.00	≤5.9

Legend. *LIS is defined as 1, being the "disease". AUC=Area under the curve; SE=Standard error; CI=Confidence interval; 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.

eTable 3. Sensitivity and specificity for different cutoff levels of ferritin

	LIS			DIU	
Used cutoff for LIS	**Sensitivity, %**	**Specificity, %**	**Used cutoff for DIU**	**Sensitivity, %**	**Specificity, %**
Ferritin <300 ng/ml & TSAT<20%	100	73	Ferritin ≥300 ng/ml & TSAT<20%	25	100
Ferritin ≤128 ng/ml & TSAT<20%	*100*	*94*	*Ferritin >128 ng/ml & TSAT<20%*	*75*	*91*
Ferritin <100 ng/ml & TSAT<20%	78	94	Ferritin ≥100 ng/ml & TSAT<20%	75	85
Ferritin <50 ng/ml & TSAT<20%	56	97	Ferritin ≥50 ng/ml & TSAT<20%	88	79
Ferritin <30 ng/ml & TSAT<20%	33	100	Ferritin ≥30 ng/ml & TSAT<20%	88	71

Legend. LIS=Low Iron Storage; DIU=Defective Iron Utilisation; TSAT=transferrin saturation.

eTable 4. Linear regression for iron biomarkers with bone marrow iron storage and iron incorporation

Variables	Iron storage		Iron incorporation	
	P-value	R-squared	P-value	R-squared
Hemoglobin, g/dl	<0.001	0.38	<0.001	0.28
Hematocrit, %	0.002	0.23	0.018	0.14
Reticulocytes, ‰	0.699	0.00	0.982	0.00
RPI	0.022	0.13	0.097	0.07
MCV, fL	0.240	0.04	0.283	0.03
MCH, fmol	0.026	0.12	0.019	0.14
MCHC, g/dl	0.005	0.19	0.001	0.25
RDW, %	0.015	0.14	0.020	0.13
HYPO, %	0.044	0.10	0.056	0.09
RET-He, pg	<0.001	0.27	<0.001	0.27
RBC-He, pg	0.011	0.16	0.017	0.14
Delta-He, pg	0.002	0.22	<0.001	0.28
Ferritin, ng/mL	**<0.001**	**0.43**	<0.001	0.30
TSAT, %	<0.001	0.26	**<0.001**	**0.49**
sTfR, mg/l	0.002	0.22	0.010	0.16
sTfR/log ferritin index	<0.001	0.38	0.001	0.23
Log Hepcidin, nM	<0.001	0.37	0.082	0.07

Legend. 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.

eTable 5. Baseline characteristics in BIOSTAT-CHF study

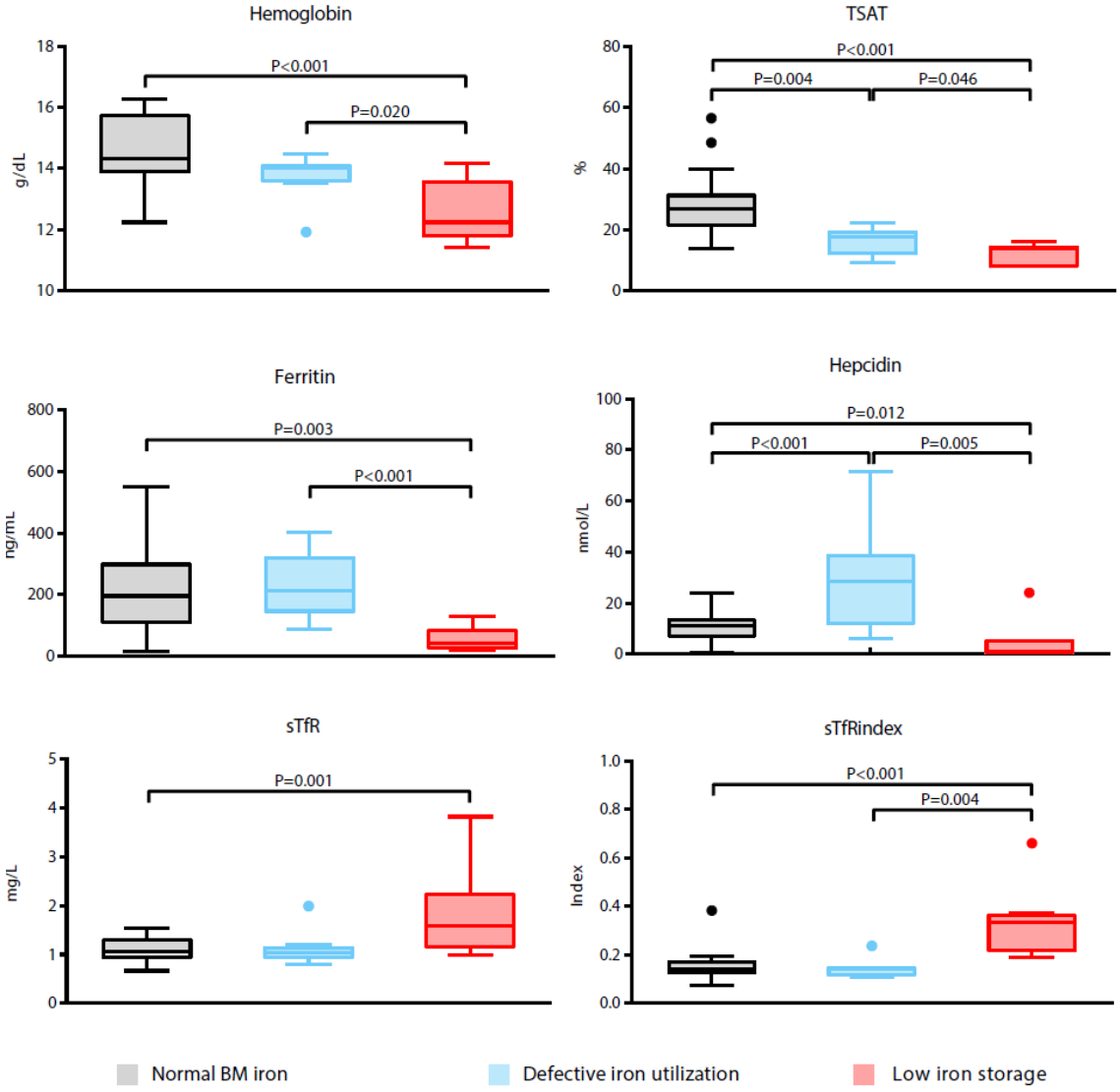
Factor	Lvl.	No ID	ID	DIU	LIS
N		904	1453	493	960
Clinical characteristics					
Age (years)		68.1 (12.1)	69.3 (11,9)	68.5 (11.9)	69.8 (11.9)
Women (%)		178 (19.7%)	438 (30.1%)	113 (22.9%)	325 (33.9%)
BMI (kg/m2)		27.8 (5.3)	27.9 (5,6)	27.8 (5.5)	28.0 (5.6)
Protein intake (g/day)		56.8 (12.2)	53.9 (10.5)	54.4 (10.5)	53.7 (10.4)
Ischemic etiology (%)		395 (44.6%)	674 (47.1%)	222 (45.4%)	452 (48.0%)
LVEF (%)		30 (25, 35)	30 (25, 37)	30 (25, 38)	30 (25, 36)
HFrEF		779 (93.6%)	1138 (89.0%)	378 (88.3%)	760 (89.4%)
NYHA functional class prior to worsening HF	I	84 (10.2%)	132 (10.6%)	60 (14.3%)	72 (8.7%)
	II	454 (55.4%)	630 (50.4%)	205 (48.7%)	425 (51.2%)
	III	253 (30.9%)	439 (35.1%)	138 (32.8%)	301 (36.3%)
	IV	29 (3.5%)	50 (4.0%)	18 (4.3%)	32 (3.9%)
Systolic blood pressure (mmHg)		124.1 (19.9)	125,0 (22,9)	124.4 (23.6)	125.2 (22.5)
Medical History					
Atrial fibrillation (%)		393 (43.5%)	670 (46.1%)	215 (43.6%)	455 (47.4%)
Diabetes mellitus (%)		238 (26.3%)	521 (35.9%)	160 (32.5%)	361 (37.6%)
Renal disease (%)		177 (19.6%)	472 (32.5%)	142 (28.8%)	330 (34.4%)
Hypertension (%)		540 (59.7%)	929 (63.9%)	315 (63.9%)	614 (64.0%)
Medication					
Loop Diuretics		902 (99.8%)	1444 (99.4%)	486 (98.6%)	958 (99.8%)
β-blocker		796 (88.1%)	1167 (80.3%)	395 (80.1%)	772 (80.4%)
ACEi/ARB		696 (77.0%)	1012 (69.6%)	346 (70.2%)	666 (69.4%)
Aldosteron antagonist		514 (56.9%)	745 (51.3%)	242 (49.1%)	503 (52.4%)
Anti-platelet therapy		458 (50.7%)	767 (52.8%)	251 (50.9%)	516 (53.8%)
Oral iron		25 (2.8%)	66 (4.5%)	21 (4.3%)	45 (4.7%)
Intravenous iron		1 (0.1%)	2 (0.1%)	0 (0.0%)	2 (0.2%)
Hematology					

Hemoglobin (g/dL)		13.9 (1.8)	12.8 (1.8)	13.2 (1.9)	12.6 (1.8)
Anemia yes/no		181 (23.0%)	597 (43.8%)	182 (38.8%)	415 (46.4%)
Hematocrit (%)		41.6 (5.2)	39.1 (5.2)	39.7 (5.6)	38.8 (5.0)
Mean corpuscular volume (fL)		92.3 (8.8)	89.4 (8.3)	91.4 (7.5)	88.2 (8.5)
Mean cell hemoglobin (pg)		30.8 (3.1)	29.3 (3.1)	30.3 (2.7)	28.7 (3.2)
Mean corpuscular hemoglobin concentration (g/dL)		33.3 (1.2)	32.8 (1.4)	33.2 (1.2)	32.6 (1.4)
Iron (µg/dL)		78.2 (61.5, 100.6)	33.5 (22.3, 44.7)	39.1 (27.9, 50.3)	33.5 (22.3, 44.7)
Ferritin (ng/mL)		142 (80, 240)	78 (39, 162)	202 (161, 290)	51 (28, 77)
Transferrin saturation (%)		27.4 (23.3, 33.2)	12.4 (8.8, 15.9)	13.9 (10.3, 17.1)	11.6 (8.1, 15.5)
Hepcidin (nmol/L)		8.4 (4.4, 20.0)	4.6 (1.4, 13.1)	14.3 (7.5, 26.8)	2.3 (0.8, 6.0)
sTfR (mg/L)		1.3 (1.0, 1.7)	1.7 (1.3, 2.3)	1.5 (1.1, 1.9)	1.8 (1.4, 2.6)
Biomarkers					
CRP (mg/L)		8.0 (3.5, 17.2)	16.9 (8.4, 32.1)	22.5 (11.9, 40.5)	14.6 (7.3, 27.4)
Leucocytes (10e9/L)		7.5 (6.3, 9.1)	8.0 (6.6, 9.8)	8.4 (6.9, 10.3)	7.8 (6.3, 9.5)
IL-6 (pg/mL)		3.2 (2.0 – 6.2)	6.5 (3.8 – 13.1)	7.9 (4.3 – 15.4)	6.1 (3.5 – 11.8)
Myeloperoxidase (ng/mL)		27.7 (23.1, 34.9)	28.5 (23.8, 35.9)	28.8 (23.8, 37.2)	28.2 (23.7, 35.4)
Procalcitonin (pg/mL)		11.8 (4.1, 28.4)	19.8 (7.3, 45.5)	22.5 (9.5, 52.7)	18.0 (6.7, 41.7)
NT-proBNP (ng/L)		3300 (1833, 6767)	4812 (2688, 8991)	5459 (2778, 9933)	4482 (2642, 8644)
eGFR (CKD-EPI) (mL/min/1.73 m2)		64.3 (47.9, 82.8)	57.2 (42.6, 76.2)	58.6 (43.4, 77.9)	56.6 (42.5, 74.6)
Creatinin (µmol/L)		97.2 (79.6, 122.0)	104.0 (84.0, 132.6)	103.2 (85.4, 134.5)	104.0 (84.0, 132.6)

Legend. 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.

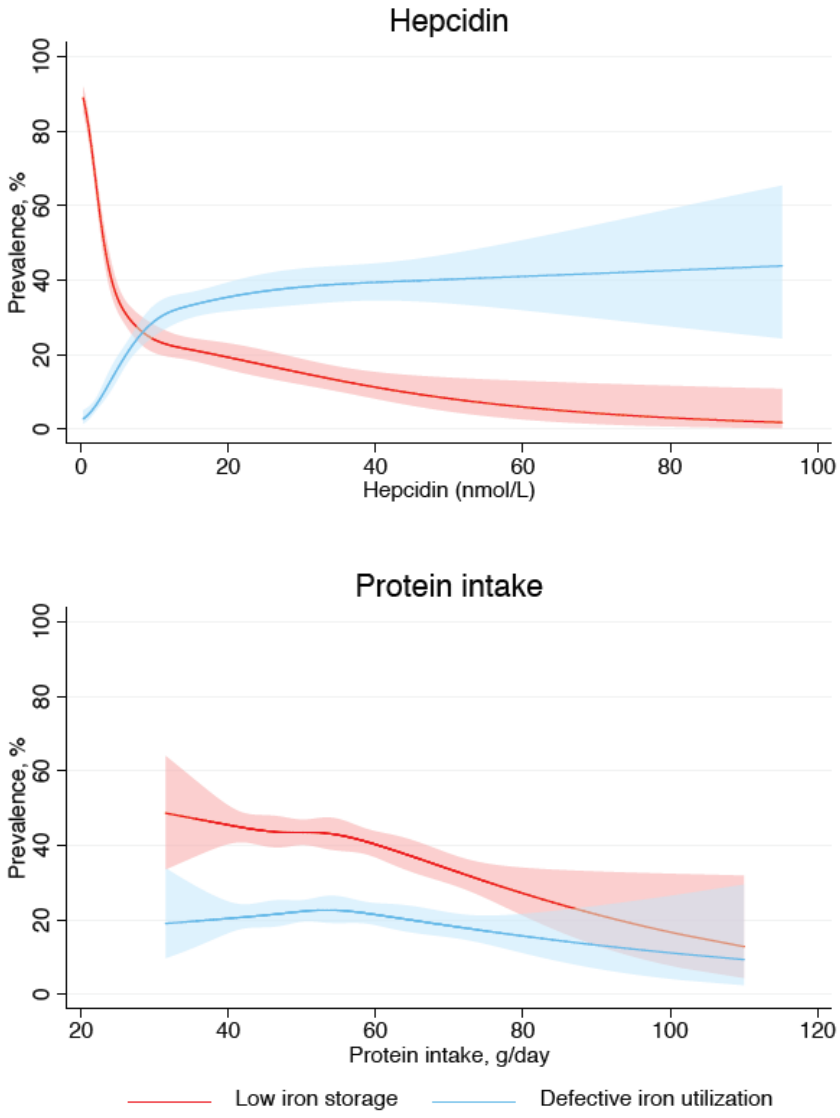
BMI=body mass index; LVEF=left ventricular ejection fraction; NYHA class=New York Heart Association class; ACE/ARB=Angiotensin converting enzyme inhibitor or angiotensin receptor blocker; sTfR=soluble transferrin receptor; CRP=c-reactive protein; NT-proBNP=N-terminal pro-brain natriuretic Peptide; eGFR=estimated glomerular filtration rate; CKD-EPI=Chronic kidney disease Epidemiology Collaboration.

eFigure 1. Levels of iron biomarkers per bone marrow iron status



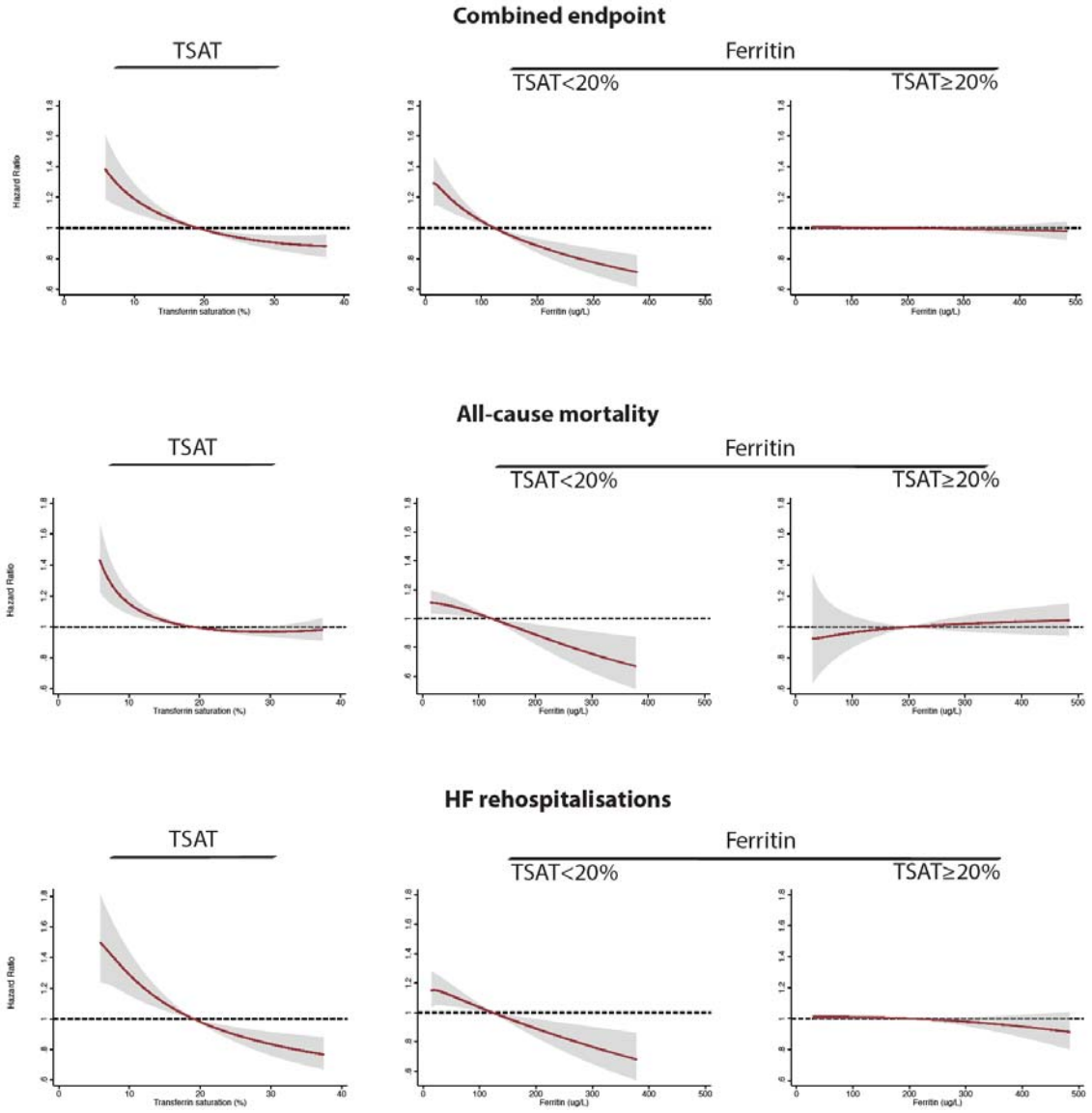
Legend. Only significant (P<0.05) differences between categories of iron status are depicted. TSAT=transferrin saturation; sTfR=soluble transferrin receptor; BM=bone marrow.

eFigure 2. Association of the prevalence of LIS and DIU with hepcidin level and protein intake



Legend. The association between prevalence of type of iron deficiency with hepcidin levels and daily protein intake depicted by restricted cubic splines. P-values depict significance of the whole model.

eFigure 3. Continuous hazard regression analysis of transferrin saturation and ferritin levels



Legend. Continuous hazard using fractional polynomials for TSAT and ferritin stratified for TSAT <20% (iron deficiency) vs. TSAT ≥20% (no iron deficiency) (all P-values for interaction<0.05). All models have been adjusted for the BIOSTAT-CHF prediction models and additionally for hemoglobin and eGFR. TSAT = transferrin saturation, HF = heart failure.

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