University of Groningen

Differences in Clinical Profile and Outcomes of Low Iron Storage vs Defective Iron Utilization in Patients With Heart Failure

Grote Beverborg, Niels; van der Wal, Haye H; Klip, IJsbrand T; Anker, Stefan D; Cleland, John; Dickstein, Kenneth; van Veldhuisen, Dirk J; Voors, Adriaan A; van der Meer, Peter

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

JAMA Cardiology | **Brief Report**

Differences in Clinical Profile and Outcomes of Low Iron Storage vs Defective Iron Utilization in Patients With Heart Failure
Results From the DEFINE-HF and BIOSTAT-CHF Studies

Niels Grote Beverborg, MD, PhD; Haye H. van der Wal, MD; IJsbrand T. Klip, MD, PhD; Stefan D. Anker, MD, PhD; John Cleland, MD, PhD; Kenneth Dickstein, MD, PhD; Dirk J. van Veldhuisen, MD, PhD; Adriaan A. Voors, MD, PhD; Peter van der Meer, MD, PhD

➕ Supplemental content

IMPORTANCE Iron deficiency is present in half of patients with heart failure (HF) and is associated with increased morbidity and an impaired prognosis. Iron deficiency due to low iron storage (LIS) and defective iron utilization (DIU) are not entirely the same clinical problem, although they generally receive the same treatment.

OBJECTIVE To define and describe similarities and differences between LIS and DIU in patients with HF.

DESIGN, SETTING, AND PARTICIPANTS This analysis included data from 2 prospective observational studies: the Definition of Iron Deficiency in Chronic Heart Failure (DEFINE-HF) study, a single-center study conducted from 2013 to 2015 including 42 patients with a reduced left ventricular ejection fraction of 45% or less scheduled for coronary artery bypass graft surgery, and the A Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) study, a multinational study conducted from 2010 to 2014 including 2357 patients with worsening HF from 69 centers in 11 countries. The median (interquartile range) follow-up time was 1.8 (1.3-2.3) years. Data were analyzed from January 2018 to January 2019.

MAIN OUTCOMES AND MEASURES The DEFINE-HF cohort was set up to derive a definition for different etiologies of iron deficiency using bone marrow iron staining as the criterion standard. This definition was applied to the BIOSTAT-CHF cohort to assess its association with clinical profile, biomarkers, and the primary composite end point of all-cause mortality or HF hospitalizations.

RESULTS Among the 42 patients in the DEFINE-HF study, 10 (24%) were women, and the mean (SD) age was 68.0 (9.5) years. Low iron storage was defined as a bone marrow–validated combination of transferrin saturation less than 20% and a serum ferritin concentration of 128 ng/mL or less; DIU was defined as transferrin saturation less than 20% and a serum ferritin concentration greater than 128 ng/mL. These criteria were applied to 2356 patients with worsening HF in the BIOSTAT-CHF study; 1074 (45.6%) were women, and the mean (SD) age was 68.9 (12.0) years. A total of 1453 patients with worsening HF (61.6%) had iron deficiency, of whom 960 (66.1%) had LIS and 493 (33.9%) had DIU. Low iron storage was characterized by a higher proportion of anemia and a poorer quality of life, while DIU was characterized by higher levels of various inflammatory markers. Both LIS and DIU were associated with an impaired 6-minute walking test. Low iron storage was independently associated with the composite end point of all-cause mortality or HF hospitalizations (hazard ratio, 1.47; 95% CI, 1.26-1.71; $P < .001$), while DIU was not (hazard ratio, 1.05; 95% CI, 0.87-1.26; $P = .64$).

CONCLUSIONS AND RELEVANCE In this study, both LIS and DIU were prevalent in patients with HF and had a distinct clinical profile. Only LIS was independently associated with increased rates of morality and HF hospitalizations, while DIU was not.

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Peter van der Meer, MD, PhD, Department of Cardiology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, PO Box 30.001, 9700 RB Groningen, the Netherlands (p.van.der.meer@umcg.nl).

ron deficiency (ID) is a prevalent comorbidity in patients with heart failure (HF) that impairs oxygen transport and oxygen use owing to its important role in hemoglobin and mitochondrial respiration.[1-4] In severely iron-deficient patients, contractile force of the myocardium may even deteriorate,[1] resulting in poor exercise capacity and quality of life and increased mortality.[5-8]

Generally, 2 distinct mechanisms of ID are distinguished. One is a shortage in the absolute number of iron molecules, often caused by an imbalance between iron uptake and loss, resulting in low iron storage (LIS) and, consequently, iron availability. In the other, in case of low-grade inflammation, hepcidin production is increased, causing iron to be trapped inside the mononuclear phagocyte system.[9] This results in defective iron utilization (DIU) and could subsequently lead to anemia of chronic disease.

Both LIS and DIU are ill-characterized in HF because of the lack of validated practical diagnostic tools at hand. However, with the use of bone marrow iron staining, it is possible to distinguish the 2 conditions.[10] Our goal was to provide a practical biomarker tool to diagnose both LIS and DIU and apply this tool in a large HF population.

Methods

Detailed methods are available in the eMethods in the Supplement. Institutional review board approval was not needed for this report because the analyses performed were included in the approved project plans of both individual studies.

Patients

Two different patient cohorts were used for this study. In the Definition of Iron Deficiency in Chronic Heart Failure (DEFINE-HF) study,[3] conducted from 2013 to 2015, we performed bone marrow iron assessment to obtain a biomarker-based definition of LIS and DIU. A total of 42 patients scheduled for coronary artery bypass graft surgery with a history of HF, a reduced left ventricular ejection fraction of 45% or less, and a plasma amino-terminal pro–B-type natriuretic peptide concentration greater than 125 pg/mL (to convert to nanograms per liter, multiply by 1) were eligible. Bone marrow aspirates were taken from the sternum during coronary artery bypass graft surgery and stained for non–heme-bound iron using the Prussian blue staining. To distinguish LIS from DIU, the amount of iron present in the extracellular space (storage) was graded using the Gale method.[11]

The second cohort was used to apply this definition and explore characteristics of patients with either LIS or DIU. In brief, the A Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) study,[12] conducted from 2010 to 2014, enrolled 2516 participants with worsening HF who either presented at the outpatient clinic or were hospitalized for worsening HF from 69 centers in 11 countries. Iron parameters were missing in 159 patients, resulting in 2357 participants available for analysis. Main inclusion criteria were left ventricular ejection fraction of 40% or less or amino-terminal pro–B-type natriuretic peptide levels greater than

Key Points

Question What are the clinical differences between iron deficiency caused by low iron storage and defective iron utilization in patients with heart failure?

Findings In this analysis of the A Systems Biology Study to Tailored Treatment in Chronic Heart Failure study, which included a total of 2357 patients, low iron storage was distinguished from defective iron utilization using a definition validated with bone marrow iron stainings in 42 patients with heart failure from the Definition of Iron Deficiency in Chronic Heart Failure study. Both conditions were prevalent and had a distinct clinical profile; only low iron storage was associated with a poor prognosis.

Meaning In patients with heart failure, distinguishing iron deficiency caused by low iron storage and defective iron utilization should be considered.

2000 pg/mL or B-type natriuretic peptide levels greater than 400 pg/mL. More details on the BIOSTAT-CHF study have been published previously.[13,14]

Both studies were approved by the local medical ethical committee at each center and complied with the Declaration of Helsinki. All participants provided written informed consent prior to any study handling.

Statistical Analyses

Data are presented as means and SDs for normally distributed continuous variables, as medians and interquartile ranges for nonnormally distributed continuous variables, or as frequencies and percentages for categorical variables. Differences between baseline variables were tested using t test for normally distributed continuous variables, Wilcoxon rank sum test (2 groups) and Kruskal-Wallis test (3 groups) for nonnormally distributed continuous variables, and Pearson χ^2 test for categorical variables. We performed receiver operator characteristic curve analysis to estimate the ability of the different markers of iron status to separate LIS from DIU. Cox proportional hazard regression analyses were performed in a univariable and multivariable model including all variables included in the BIOSTAT-CHF prediction models and additionally corrected for hemoglobin level and estimated glomerular filtration rate.[14] We considered a 2-sided P value less than .05 statistically significant and a P values less than .10 significant for interaction analyses. All tests and analyses were performed using Stata version 15.0 (StataCorp) and GraphPad Prism version 5.04 (GraphPad Software).

Results

Definition of LIS and DIU Using Bone Marrow Iron Staining

Baseline characteristics of the 42 patients with HF from the DEFINE-HF study[3] in whom bone marrow iron staining was performed are presented in eTable 1 and eFigure 1 in the Supplement. Of the 17 patients with bone marrow ID, 8 had DIU and 9 LIS. Receiver operating characteristic results are depicted in eTable 2 in the Supplement. We found the highest area under

the curve (SE) for the discrimination of LIS from DIU using ferritin level (0.97 [0.04]), the soluble transferrin receptor–ferritin index (0.97 [0.04]), and hepcidin level (0.97 [0.04]). Because soluble transferrin receptor and hepcidin levels are often unavailable in clinical practice, we selected ferritin levels as the diagnostic criterion for LIS (optimal cutoff value of 128 ng/mL [to convert to picomoles per liter, multiply by 2.247] or less) (**Figure 1**A). This definition had a sensitivity of 100% and specificity of 94% for LIS vs no LIS and a sensitivity of 75% and specificity of 91% for DIU vs no DIU (Figure 1B). See eTable 3 in the Supplement for comparison with other ferritin cutoff values. Continuous linear regression results between either iron storage or iron incorporation with iron biomarkers is depicted in eTable 4 in the Supplement. Iron availability, the discriminator between ID and no ID, is best predicted by TSAT (R^2, 0.49; $P < .001$), while iron storage, the discriminator between LIS and DIU, is best predicted by ferritin levels (R^2, 0.43; $P < .001$), supporting the receiver operating characteristic results.

Patient Characteristics in BIOSTAT-CHF Cohort

Baseline characteristics of the BIOSTAT-CHF cohort[12] are presented in eTable 5 in the Supplement. Of a total of 2357 patients, 1453 patients (61.6%) had ID using the validated cutoff of TSAT less than 20%. Of these, when applying the ferritin cutoff value of 128 ng/mL, 960 (66.1%) had LIS and 493 (33.9%) had DIU (Figure 1C). Higher hepcidin levels were significantly associated with a lower prevalence of LIS and a higher prevalence of DIU (eFigure 2 in the Supplement). Daily protein intake was lower in patients with ID, and a lower protein intake was significantly associated with a higher incidence of LIS but not DIU (eFigure 2 in the Supplement). Overall, the treatment rate with oral or intravenous iron was low (3.9% and 0.1%, respectively) and did not differ between the LIS and DIU groups. No patient used erythropoiesis-stimulating agents. Iron deficiency was independently associated with signs and symptoms of HF, inflammation, anemia, and renal failure (**Figure 2**). Interaction analysis revealed that LIS was independently associated with a higher prevalence of right-sided congestion, anemia, and lower quality of life, while DIU was associated with higher incidences of increased parameters of inflammation (C-reactive protein and interleukin 6 levels).

Prognosis

Median (interquartile range) follow-up was 1.8 (1.3-2.3) years, and over a total of 2.8 years follow-up, 400 patients (22.3%) died and 394 (22.0%) were hospitalized for HF. The presence of ID was independently associated with the composite end point of all-cause mortality and HF hospitalization (hazard ratio, 1.31; 95% CI, 1.13-1.51; $P < .001$) and its individual components (**Table**). We observed an interaction between LIS and DIU on all end points. Low iron storage was significantly and independently associated with worse prognosis (hazard ratio, 1.47; 95% CI, 1.26-1.71; $P < .001$), while DIU was not (hazard ratio, 1.05; 95% CI, 0.87-1.26; $P = .64$). These results were confirmed by continuous hazard regression analysis (eFigure 3 in the Supplement), which showed low TSAT (the discriminator for ID) to be associated with unfavorable prognosis. In patients with ID, a low ferritin level (the discriminator for LIS)
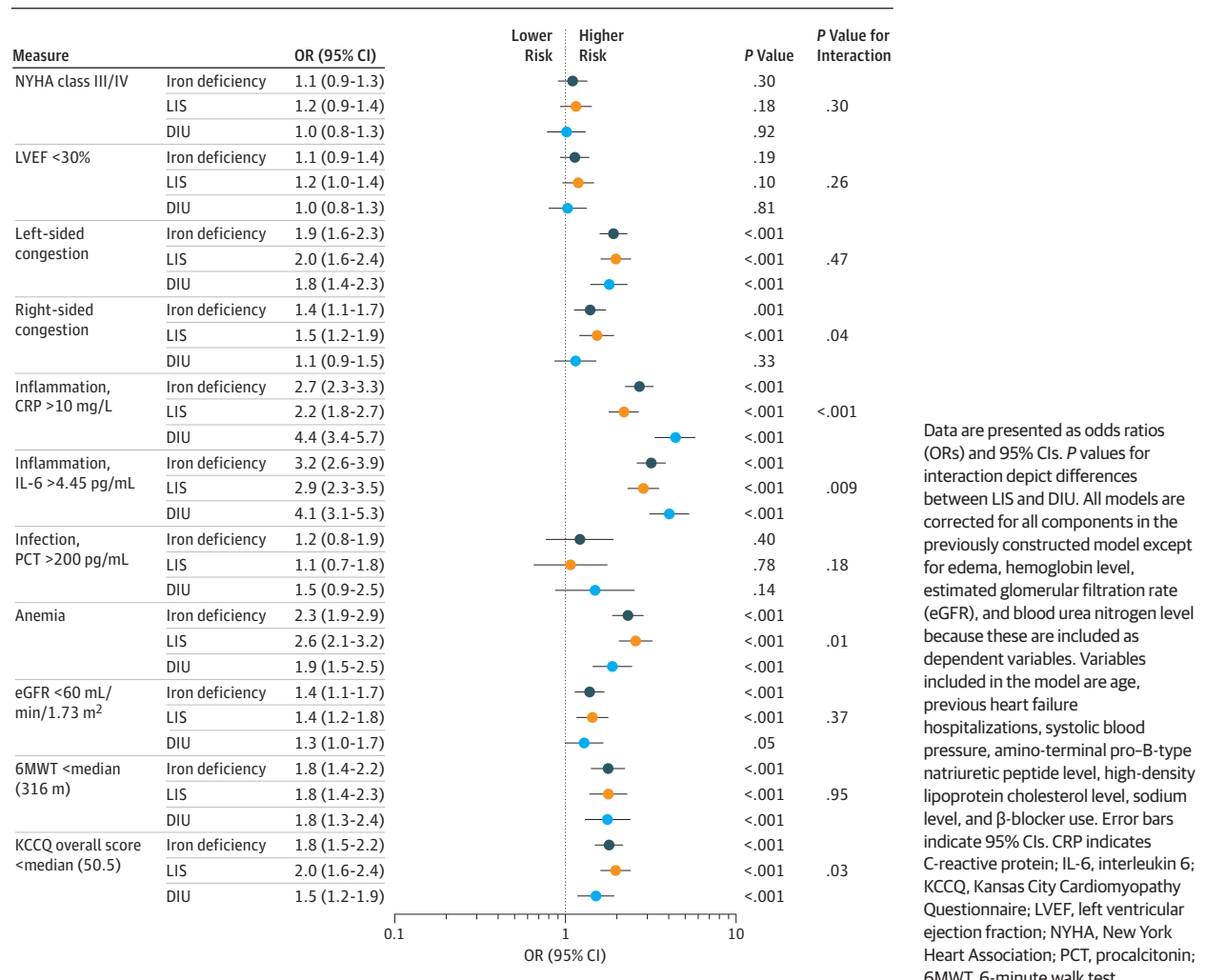
Figure 1. Low Iron Storage (LIS) and Defective Iron Utilization (DIU)
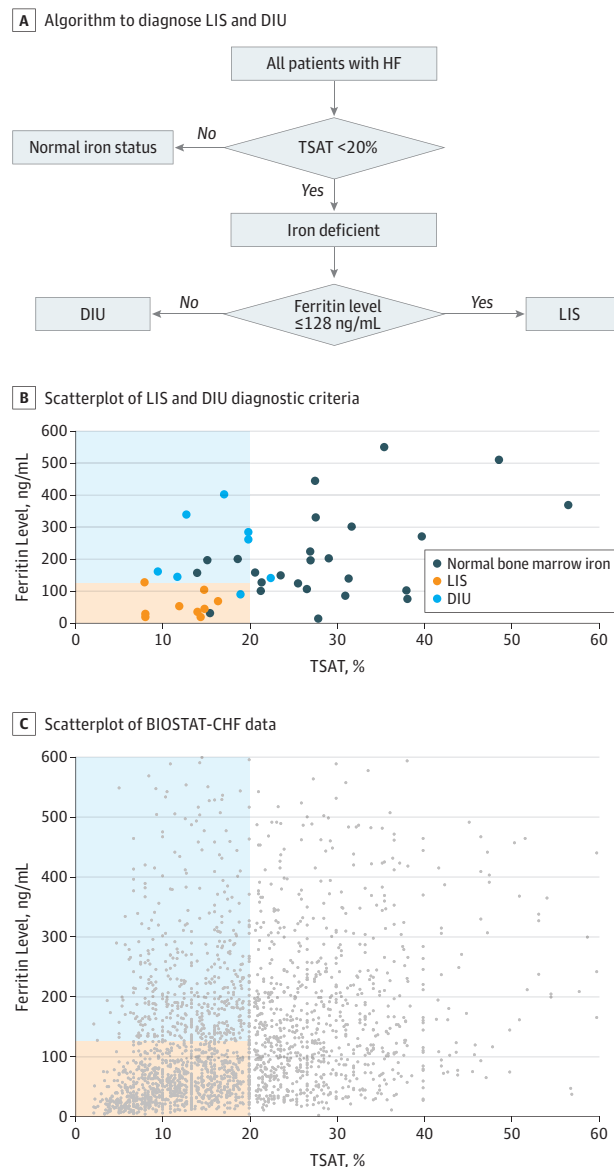
A, The algorithm to diagnose LIS and DIU obtained using the bone marrow iron staining data from 42 patients with heart failure (HF) in the Definition of Iron Deficiency in Chronic Heart Failure (DEFINE-HF) study.[3] B, Scatterplot of data from the DEFINE-HF study.[3] Each dot represents 1 patient, with black dots indicating those with normal bone marrow iron status; orange dots, those with LIS; and blue dots, those with DIU. The orange area represents the diagnostic criteria used for LIS (transferrin saturation [TSAT] <20% and ferritin level ≤128 ng/mL [to convert to picomoles per liter, multiply by 2.247]; sensitivity, 100%; specificity, 94%), and the blue area represents the diagnostic criteria used for DIU (TSAT <20% and ferritin level >128 ng/mL; sensitivity, 75%; specificity, 91%). C, Scatterplot of data from the A Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) study[12] fit to the LIS and DIU diagnostic criteria. A total of 904 patients (38.5%) had normal iron status, 960 (40.7%) had LIS, and 493 (20.9%) had DIU. Compared with patients with normal iron status, those with LIS were more often women, had lower hemoglobin levels, and higher morbidity and mortality, and those with DIU had more inflammation, higher hepcidin levels, and similar morbidity and mortality compared with patients without ID.

was associated with worse prognosis, while this was not the case in patients without ID.

Figure 2. Clinical Associates of Low Iron Storage (LIS) and Defective Iron Utilization (DIU) in the A Systems Biology Study to Tailored Treatment in Chronic Heart Failure Cohort

Measure		OR (95% CI)	P Value	P Value for Interaction
NYHA class III/IV	Iron deficiency	1.1 (0.9-1.3)	.30	
	LIS	1.2 (0.9-1.4)	.18	.30
	DIU	1.0 (0.8-1.3)	.92	
LVEF <30%	Iron deficiency	1.1 (0.9-1.4)	.19	
	LIS	1.2 (1.0-1.4)	.10	.26
	DIU	1.0 (0.8-1.3)	.81	
Left-sided congestion	Iron deficiency	1.9 (1.6-2.3)	<.001	
	LIS	2.0 (1.6-2.4)	<.001	.47
	DIU	1.8 (1.4-2.3)	<.001	
Right-sided congestion	Iron deficiency	1.4 (1.1-1.7)	.001	
	LIS	1.5 (1.2-1.9)	<.001	.04
	DIU	1.1 (0.9-1.5)	.33	
Inflammation, CRP >10 mg/L	Iron deficiency	2.7 (2.3-3.3)	<.001	
	LIS	2.2 (1.8-2.7)	<.001	<.001
	DIU	4.4 (3.4-5.7)	<.001	
Inflammation, IL-6 >4.45 pg/mL	Iron deficiency	3.2 (2.6-3.9)	<.001	
	LIS	2.9 (2.3-3.5)	<.001	.009
	DIU	4.1 (3.1-5.3)	<.001	
Infection, PCT >200 pg/mL	Iron deficiency	1.2 (0.8-1.9)	.40	
	LIS	1.1 (0.7-1.8)	.78	.18
	DIU	1.5 (0.9-2.5)	.14	
Anemia	Iron deficiency	2.3 (1.9-2.9)	<.001	
	LIS	2.6 (2.1-3.2)	<.001	.01
	DIU	1.9 (1.5-2.5)	<.001	
eGFR <60 mL/min/1.73 m²	Iron deficiency	1.4 (1.1-1.7)	<.001	
	LIS	1.4 (1.2-1.8)	<.001	.37
	DIU	1.3 (1.0-1.7)	.05	
6MWT <median (316 m)	Iron deficiency	1.8 (1.4-2.2)	<.001	
	LIS	1.8 (1.4-2.3)	<.001	.95
	DIU	1.8 (1.3-2.4)	<.001	
KCCQ overall score <median (50.5)	Iron deficiency	1.8 (1.5-2.2)	<.001	
	LIS	2.0 (1.6-2.4)	<.001	.03
	DIU	1.5 (1.2-1.9)	<.001	

Data are presented as odds ratios (ORs) and 95% CIs. P values for interaction depict differences between LIS and DIU. All models are corrected for all components in the previously constructed model except for edema, hemoglobin level, estimated glomerular filtration rate (eGFR), and blood urea nitrogen level because these are included as dependent variables. Variables included in the model are age, previous heart failure hospitalizations, systolic blood pressure, amino-terminal pro–B-type natriuretic peptide level, high-density lipoprotein cholesterol level, sodium level, and β-blocker use. Error bars indicate 95% CIs. CRP indicates C-reactive protein; IL-6, interleukin 6; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PCT, procalcitonin; 6MWT, 6-minute walk test.

Discussion

Low iron storage and DIU in patients with HF are generally treated similarly. However, in the present study, we demonstrated important differences. Using bone marrow–validated data, LIS could be distinguished from DIU when serum ferritin concentrations were less than 128 ng/mL. Low iron storage was independently associated with a higher prevalence of anemia and poorer quality of life, while DIU was associated with a higher incidence of inflammation. Low iron storage, but not DIU, was independently associated with higher mortality risk.

Definition of ID in Patients With HF

In 2018, we proposed a definition of ID in patients with HF based on bone marrow iron staining,[3] which is considered the criterion standard. Transferrin saturation less than 20% was shown to be optimal for the diagnosis of ID and selected patients with the worst prognosis and patients that responded best to intravenous ferric carboxymaltose in terms of improved prognosis in a post hoc analysis.[3] In the current study, we applied this definition to a large population of patients with worsening HF. Iron deficiency was shown to be prevalent (62%), associated with more signs and symptoms, and independently associated with worse prognosis.

LIS and DIU

Diagnostics

Differentiating LID from DIU ideally requires bone marrow iron staining.[10] However, this examination is painful and often unsuccessful. Circulating biomarkers are more applicable in clinical practice. In our relatively small study, a ferritin level of 128 ng/mL or less was optimal to diagnose bone marrow–defined LIS. This is in reasonable agreement with the cutoff values reported in patients with chronic kidney disease (100 ng/mL) and coronary artery disease (112 ng/mL).[10,15]

Association of ID With Morbidity and Mortality

In this study, LIS was strongly associated with increased all-cause mortality and HF hospitalization rates, while DIU was not. These data raise the question if patients with DIU should be treated with intravenous iron with the goal of improving

jamacardiology.com

Table. Prognosis of Patients According to Iron Status

End Point	Iron Status	Univariable		Multivariable[a]		P Value for Interaction[b]
		HR (95% CI)	P Value	HR (95% CI)	P Value	
Composite end point	No ID	1 [Reference]	NA	1 [Reference]	NA	
	ID	1.68 (1.46-1.93)	<.001	1.31 (1.13-1.51)	<.001	
	LIS	1.87 (1.62-2.17)	<.001	1.47 (1.26-1.71)	<.001	<.001
	DIU	1.34 (1.12-1.61)	.002	1.05 (0.87-1.26)	.64	
HF hospitalization	No ID	1 [Reference]	NA	1 [Reference]	NA	
	ID	1.68 (1.41-2.01)	<.001	1.49 (1.24-1.80)	<.001	
	LIS	1.83 (1.51-2.21)	<.001	1.64 (1.36-1.99)	<.001	.07
	DIU	1.41 (1.11-1.78)	.004	1.32 (1.04-1.68)	.02	
All-cause mortality	No ID	1 [Reference]	NA	1 [Reference]	NA	
	ID	1.63 (1.37-1.94)	<.001	1.24 (1.04-1.49)	.02	
	LIS	1.80 (1.49-2.17)	<.001	1.37 (1.13-1.66)	.001	.006
	DIU	1.32 (1.04-1.66)	.02	1.02 (0.80-1.29)	.89	

Abbreviations: DIU, defective iron utilization; HF, heart failure; HR, hazard ratio; ID, iron deficiency; LIS, low iron storage; NA, not applicable.

[a] Corrected for the A Systems Biology Study to Tailored Treatment in Chronic Heart Failure prediction model, with additional correction for hemoglobin and glomerular filtration rate in all models. Composite end point adjusted for age, previous HF hospitalization, edema, systolic blood pressure, natural log–transformed amino-terminal pro–B-type natriuretic peptide, hemoglobin level, high-density lipoprotein level, sodium level, β-blocker use, and estimated glomerular filtration rate. Heart failure hospitalizations adjusted for age, previous HF hospitalization, edema, systolic blood pressure, and estimated glomerular filtration rate. All-cause mortality adjusted for age, natural log–transformed blood urea nitrogen level, natural log–transformed amino-terminal pro–B-type natriuretic peptide, hemoglobin level, β-blocker use, and estimated glomerular filtration rate.

[b] P value for interaction tests depicts differences between LIS and DIU.

prognosis. After all, if DIU does not affect prognosis, could we expect any survival benefit from treating the disorder? Although our findings are hypothesis generating and definitely need confirmation, they might suggest the implementation of a prespecified subgroup analysis in outcome trials currently being conducted.

Strengths and Limitations

We assessed iron status using the criterion standard in which we took both iron storage and iron incorporation into consideration to define LIS and DIU in patients with HF. A limitation is the size and design of the bone marrow study. We included only patients with HF with a reduced ejection fraction from 1 tertiary center who were scheduled for coronary artery bypass graft surgery, limiting generalizability. Consequently, the application of the diagnostic criteria to the BIOSTAT-CHF cohort[12] is not optimal, and our bone marrow data require validation in an independent cohort of patients with HF.

Conclusions

In patients with HF, both LIS and DIU are associated with a poorer exercise capacity and quality of life compared with patients without ID. However, only LIS and not DIU was associated with an increased risk of death or HF hospitalization. Our data indicate that it might be clinically relevant to distinguish LIS from DIU in patients with HF.

Author Contributions: Dr Grote Beverborg had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Grote Beverborg, van der Wal, Klip, Anker, Cleland, Dickstein, Voors, van der Meer.
Acquisition, analysis, or interpretation of data: Grote Beverborg, van der Wal, Klip, Cleland, Dickstein, van Veldhuisen, Voors, van der Meer.
Drafting of the manuscript: Grote Beverborg, Klip, van der Meer.
Critical revision of the manuscript for important intellectual content: Grote Beverborg, van der Wal, Anker, Cleland, Dickstein, van Veldhuisen, Voors, van der Meer.
Statistical analysis: Grote Beverborg, van der Wal.
Obtained funding: Cleland, Dickstein, Voors, van der Meer.
Administrative, technical, or material support: Grote Beverborg, van der Wal, Klip, Dickstein, Voors, van der Meer.
Study supervision: Klip, Cleland, van Veldhuisen, Voors, van der Meer.

Research   Brief Report

Differences in Clinical Profile and Outcomes of Low Iron Storage vs Defective Iron Utilization in Patients With Heart Failure

and an unrestricted grant from Vifor Pharma. Dr van der Meer has grants from Vifor Pharma and personal fees from Vifor Pharma, AstraZeneca, Biotronik, and Novartis. No other disclosures were reported.

REFERENCES

1. Hoes MF, Grote Beverborg N, Kijlstra JD, et al. Iron deficiency impairs contractility of human cardiomyocytes through decreased mitochondrial function. *Eur J Heart Fail*. 2018;20(5):910-919. doi:10.1002/ejhf.1154

2. Klip IT, Comin-Colet J, Voors AA, et al. Iron deficiency in chronic heart failure: an international pooled analysis. *Am Heart J*. 2013;165(4):575-582.e3. doi:10.1016/j.ahj.2013.01.017

3. Grote Beverborg N, Klip IT, Meijers WC, et al. Definition of iron deficiency based on the gold standard of bone marrow iron staining in heart failure patients. *Circ Heart Fail*. 2018;11(2):e004519. doi:10.1161/CIRCHEARTFAILURE.117.004519

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(9):485-493. doi:10.1038/nrcardio.2011.77

5. van Veldhuisen DJ, Ponikowski P, van der Meer P, et al; EFFECT-HF Investigators. Effect of ferric

carboxymaltose on exercise capacity in patients with chronic heart failure and iron deficiency. *Circulation*. 2017;136(15):1374-1383. doi:10.1161/CIRCULATIONAHA.117.027497

6. Anker SD, Comin Colet J, Filippatos G, et al; FAIR-HF Trial Investigators. Ferric carboxymaltose in patients with heart failure and iron deficiency. *N Engl J Med*. 2009;361(25):2436-2448. doi:10.1056/NEJMoa0908355

7. Ponikowski P, van Veldhuisen DJ, Comin-Colet J, et al; CONFIRM-HF Investigators. 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(11):657-668. doi:10.1093/eurheartj/ehu385

8. Lewis GD, Malhotra R, Hernandez AF, et al; NHLBI Heart Failure Clinical Research Network. Effect of oral iron repletion on exercise capacity in patients with heart failure with reduced ejection fraction and iron deficiency: the IRONOUT HF randomized clinical trial. *JAMA*. 2017;317(19):1958-1966. doi:10.1001/jama.2017.5427

9. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med*. 2005;352(10):1011-1023. doi:10.1056/NEJMra041809

10. Thomas DW, Hinchliffe RF, Briggs C, Macdougall IC, Littlewood T, Cavill I; British Committee for Standards in Haematology.

Guideline for the laboratory diagnosis of functional iron deficiency. *Br J Haematol*. 2013;161(5):639-648. doi:10.1111/bjh.12311

11. Gale E, Torrance J, Bothwell T. The quantitative estimation of total iron stores in human bone marrow. *J Clin Invest*. 1963;42:1076-1082. doi:10.1172/JCI104793

12. Voors AA, Anker SD, Cleland JG, et al. A Systems Biology Study to Tailored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail*. 2016;18(6):716-726. doi:10.1002/ejhf.531

13. Ouwerkerk W, Voors AA, Anker SD, et al. Determinants and clinical outcome of uptitration of ACE-inhibitors and beta-blockers in patients with heart failure: a prospective European study. *Eur Heart J*. 2017;38(24):1883-1890. doi:10.1093/eurheartj/ehx026

14. Voors AA, Ouwerkerk W, Zannad F, et al. Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. *Eur J Heart Fail*. 2017;19(5):627-634. doi:10.1002/ejhf.785

15. Jankowska EA, Wojtas K, Kasztura M, et al. Bone marrow iron depletion is common in patients with coronary artery disease. *Int J Cardiol*. 2015;182:517-522. doi:10.1016/j.ijcard.2014.10.006