Association Between Norepinephrine Levels and Abnormal Iron Status in Patients With Chronic Heart Failure: Is Iron Deficiency More Than a Comorbidity?

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Background—Mechanisms underlying iron homeostasis dysregulation in patients with chronic heart failure remain unsettled. In cardiomyocyte models, norepinephrine may lead to intracellular iron depletion, but the potential association between catecholamines (sympathetic activation markers) and iron metabolism biomarkers in chronic heart failure is unknown.

Methods and Results—In this cross-sectional analysis, we studied the association between plasma norepinephrine levels and serum iron status biomarkers indicating iron storage (ferritin), iron transport (transferrin saturation), and iron demand (soluble transferrin receptor) in a prospective cohort of 742 chronic heart failure patients (mean age, 72 ± 11 years; 56% male). Impaired iron status was defined as ferritin < 100 μg/L or transferrin saturation < 20%. Impaired iron status was observed in 69% of patients. In multivariate models, greater norepinephrine levels were associated with impaired iron transport (transferrin saturation < 20%, odds ratio = 2.28; 95% CI [1.19–4.35]; P = 0.013), but not with impaired iron storage (ferritin < 100 μg/L, odds ratio = 1.25; 95% CI [0.73–2.16]; P = 0.415). Norepinephrine was a significant predictor of increased iron demand (soluble transferrin receptor, standardized β-coefficient = 0.12; P = 0.006) and low transferrin saturation (standardized β-coefficient = –0.12; P = 0.003). However, norepinephrine levels were not associated with iron or ferritin levels (P > 0.05). Adjusted norepinephrine marginal means were significantly higher in patients with impaired iron status compared with those with normal iron status (528 pg/mL [505–551] versus 482 pg/mL [448–518], respectively; P = 0.038).

Conclusions—In chronic heart failure patients, increased sympathetic activation estimated with norepinephrine levels is associated with impaired iron status and, particularly, dysregulation of biomarkers suggesting impaired iron transport and increased iron demand. Whether the relationship between norepinephrine and iron metabolism is bidirectional and entails causality need to be elucidated in future research. (J Am Heart Assoc. 2019;8:e010887. DOI: 10.1161/JAHA.118.010887.)

Key Words: anemia • chronic heart failure • iron • iron deficiency • norepinephrine • sympathetic nervous system

Iron deficiency is associated with worse quality of life, functional capacity, and prognosis in patients with chronic heart failure (CHF), regardless of the presence of anemia.1–6 Consequently, current guidelines recommend assessing the iron profile of patients with CHF and treating them with intravenous iron, when appropriate.7–10 Nevertheless, the

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Clinical Perspective

What Is New?
- The exact mechanisms by which chronic heart failure patients develop iron deficiency are still unknown.
- To the best of our knowledge, we demonstrate, for the first time, an interplay between increased sympathetic nervous system activity and systemic iron deficiency in patients with chronic heart failure and, particularly, with those biomarkers that suggest impaired iron transport (transferrin saturation <20%) and increased iron demand (raised soluble transferrin receptor levels).

What Are the Clinical Implications?
- Our results may support the hypothesis that iron deficiency might not just be a comorbidity, but may also be a key element in the pathophysiological sequence leading to, and promoting the progression of, chronic heart failure.

Mechanisms underlying dysregulation of iron homeostasis in patients with CHF are still not well established.

Sympathetic activation, which entails increased serum levels of norepinephrine, is one of the main therapeutic targets in CHF because of its deleterious long-term effects, particularly in patients with reduced left ventricle ejection fraction (LVEF). In this context, recent studies have shown that in cardiomyocyte models, norepinephrine leads to intracellular iron depletion. These results in cellular models suggest a role of sympathetic activation in the development of iron homeostasis dysregulation. However, the association between sympathetic activity and iron status has not been evaluated so far at the patient level.

The aim of this study was thus to assess the potential association between sympathetic activation, as measured using plasma norepinephrine levels, and several biomarkers of serum iron homeostasis, including ferritin as a maker of iron availability, transferrin saturation (TSAT) as a marker of iron supply, and the soluble transferrin receptor (sTfR) as a marker of iron demand, in a large population of consecutive patients with CHF followed in a specialized, multidisciplinary CHF unit, in which all these measurements were available.

Methods

Data are available upon request from a third party. Because of the sensitive nature of the data collected for this study, reasonable requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to Bellvitge Biomedical Research Institute at the corresponding author.

Study Design and Study Population

The DAMOCLES (Definition of the neuro-hormonal activation, myocardial function, genomic expression and clinical outcomes in heart failure patients) study was a single-center, observational, prospective cohort study of 1236 consecutive patients diagnosed with CHF. The methodology of the DAMOCLES has been published previously by our group. Briefly, for inclusion, patients had to be diagnosed with CHF according to the European Society of Cardiology diagnostic criteria, had at least 1 recent acute decompensation of CHF requiring intravenous diuretic therapy (either hospitalized or in the day care hospital), and had to be in stable condition at the time of study entry. Exclusion criteria were: significant primary valvular disease, clinical signs of fluid overload, pericardial disease, restrictive cardiomyopathy, hypertrophic cardiomyopathy, hemoglobin levels <8.5 g/dL, active malignancy, and chronic liver disease. The study was approved by the local committee of ethics for clinical research and was conducted in accord with the principles of the Declaration of Helsinki. All patients gave written informed consent before study entry.

For the present analysis, norepinephrine levels were evaluated in all consecutive participants included in the DAMOCLES study between June 2004 and January 2011. Patients included in the DAMOCLES study after this period did not have norepinephrine levels measured and thus were excluded from this analysis.

Baseline Evaluation: Catecholamines, Iron Parameters, and Other Laboratory Measurements

Methods of blood collection and management, as well as the quantification of norepinephrine levels performed in the baseline DAMOCLES study visit, have been previously reported. In summary, patients were resting in a supine position in a quiet room for 30 to 60 minutes after venous cannulation. Tubes with blood samples were immersed in melting ice and frozen until they were processed. Levels of norepinephrine were measured from 1.5 mL of plasma by high-resolution liquid chromatography. Norepinephrine analysis had a coefficient of variation of 8.7%. Serum N-terminal pro-B-type natriuretic peptide levels were measured in pg/mL using an immunoassay based on chemiluminescence using the Elecsys System (Roche, Indianapolis, IN). Serum iron (mg/dL) was measured using spectrophotometry; serum ferritin (ng/mL) and transferrin (mg/dL) were measured using immunoturbidimetry. TSAT was estimated using the formula: TSAT = serum iron (mg/dL)/[serum transferrin (mg/dL) × 1.25]. Iron status was also assessed by measuring serum soluble transferrin receptor (sTfR; in mg/L) levels using an enzyme immunoassay. Hemoglobin (g/dL) was measured with
impedance laser colorimetry. Glomerular filtration rate was estimated from serum creatinine using the formula of the Modification of Diet in Renal Disease Study Group equation.\textsuperscript{18}

### Study Definitions

Impaired iron status was defined as ferritin $<100$ ng/mL or TSAT $<20\%$. To explore the association between norepinephrine and different components of the iron pathway, we also defined impaired iron storage as ferritin $<100$ ng/mL and impaired iron transport as TSAT $<20\%$. Increased iron demand (high sTfR) was defined as levels of sTfR $>75$th percentile of its distribution in the study population. Anemia was defined using the World Health Organization criteria (cut-off values of 13 g/dL in men and 12 g/dL in women).\textsuperscript{19}

### Statistical Analyses

This was a cross-sectional analysis using the baseline data from the DAMOCLES study. Demographic and clinical characteristics and laboratory test results were summarized using basic descriptive statistics, both in the overall cohort as well as stratified by norepinephrine tertiles. For quantitative variables, the arithmetic mean (and SD) or median (and interquartile range) were reported as appropriate, and $P$ values were obtained using ANOVA and Kruskal–Wallis tests, respectively. For qualitative variables, number and percentages within specified groups were calculated, and $P$ values were derived using $\chi^2$ tests.

When necessary, natural logarithm transformation was used to fit skewed continuous variables into normal distributions. Specifically, norepinephrine was modeled in 2 ways for the analyses: as a continuous exposure (log-transformed), as well as a dichotomous exposure (“high norepinephrine” levels defined as norepinephrine $>90$th percentile [1050 pg/mL] of the distribution in the study population, as compared with $<90$th percentile).

To assess the association between sympathetic activation, as measured using norepinephrine levels, and the different iron status biomarkers, we first used logistic and linear regression to assess the crude (unadjusted) associations between norepinephrine and each of the relevant iron status biomarkers. Also, generalized additive models were used to graphically display the relationship between log norepinephrine serum levels and log TSAT, log sTfR, and log ferritin, respectively. Unadjusted logistic and linear regression analyses were also used to assess the bivariate associations between each of the predictors included in Table 1 and each of the iron status biomarkers.

Multivariable-adjusted linear and logistic regression models were used to evaluate the adjusted associations between higher levels of norepinephrine as an independent variable and levels of each of the iron status biomarkers as the dependent variables. Multivariable models used step-wise forward conditional methods and were adjusted for the predictors that showed a significant association with impaired iron status in the bivariate logistic regression analyses.

Finally, general linear models were used to calculate adjusted marginal means and 95\% CIs of norepinephrine according to different iron and anemia states. All general linear models were adjusted for factors associated with increased norepinephrine levels. Factors associated with raised norepinephrine levels have been described previously by our group.\textsuperscript{17}

All statistical tests and CIs were constructed with a type 1 error alpha level of 5\% with no adjustments for multiplicity, and $P<0.05$ were considered statistically significant. SPSS (version 22.0; IBM, Armonk, NY) and R software (version 3.0.1; R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analyses.

### Results

#### Study Population

Of the 1236 CHF patients included in the DAMOCLES study, 494 did not have baseline levels of norepinephrine measured and were excluded from the present analyses. This defined a final study population of 742 patients. Serum iron status parameters were available in all DAMOCLES study participants.

#### Baseline Characteristics of the Study Participants

Baseline characteristics of the study participants, overall and by norepinephrine tertiles, are shown in Table 1. Overall, mean age was 72 years, and 56\% participants were male. Mean LVEF was 44\%, median N-terminal pro-b-type natriuretic peptide levels were 1547 pg/mL, and 45\% of patients had a New York Heart Association functional class of III or IV. Median levels of norepinephrine were 523 (351–730), and 515 (69\%) patients had impaired iron status.

By norepinephrine tertiles, patients in the upper tertile of norepinephrine (higher sympathetic activation) were older, more frequently male, and had a worse CHF clinical profile, with higher N-terminal pro-b-type natriuretic peptide levels, lower body mass index, and worse New York Heart Association functional class than their lower norepinephrine tertile counterparts. Also, the higher the norepinephrine tertile, the higher the prevalence of all iron abnormalities. Interestingly, LVEF, systolic blood pressure, and heart rate did not differ between categories of norepinephrine levels.
Table 1. Baseline Characteristics of the Study Population (N=742), Overall and by Norepinephrine Tertiles

| Characteristic                      | Overall  | T1 (<414 pg/mL) | T2 (414–654 pg/mL) | T3 (>654 pg/mL) | P Value |
|-------------------------------------|----------|----------------|-------------------|----------------|---------|
| Age, y                              | 72±11    | 69±11          | 73±11             | 75±11          | <0.001  |
| Sex, male, n (%)                    | 418 (56) | 137 (55)       | 134 (54)          | 147 (60)       | 0.40    |
| BMI, kg/m²                          | 28±6     | 30±6           | 28±5              | 27±5           | <0.001  |
| Systolic BP, mm Hg                  | 125±23   | 127±24         | 124±24            | 123±22         | 0.13    |
| Heart rate, bpm                     | 74±15    | 73±15          | 75±15             | 74±16          | 0.62    |
| NT-proBNP, pg/mL                    | 1547 [662–3942] | 1124 [489–2460] | 1672 [647–3495]  | 2391 [923–6436] | <0.001  |
| eGFR, mL/min per 1.73 m²            | 59±24    | 61±24          | 59±23             | 56±26          | 0.08    |
| LVEF, %                             | 44±17    | 45±17          | 44±17             | 43±18          | 0.46    |
| HfPEF, n (%)                        | 278 (38) | 91 (37)        | 95 (38)           | 92 (37)        | 0.933   |
| HfMR EF, n (%)                      | 116 (16) | 45 (18)        | 38 (15)           | 33 (13)        | 0.346   |
| HfRF EF, n (%)                      | 348 (47) | 112 (45)       | 115 (47)          | 121 (49)       | 0.655   |
| Etiology of CHF, ischemic, n (%)    | 303 (41) | 112 (45)       | 96 (39)           | 95 (39)        | 0.24    |
| Atrial fibrillation, n (%)          | 234 (32) | 72 (29)        | 85 (34)           | 77 (31)        | 0.45    |
| NYHA class III or IV, n (%)         | 330 (45) | 98 (40)        | 104 (42)          | 128 (52)       | 0.012   |
| Norepinephrine, median (IQR)        | 523 [351–730] | 313 [252–352]  | 524 [467–592]     | 858 [730–1111] | <0.001  |

Comorbidities, n (%)

| Comorbidity                          | Overall  | T1 (<414 pg/mL) | T2 (414–654 pg/mL) | T3 (>654 pg/mL) | P Value |
|--------------------------------------|----------|----------------|-------------------|----------------|---------|
| Hypertension                         | 582 (78) | 190 (77)       | 195 (79)          | 197 (80)       | 0.64    |
| Diabetes mellitus                    | 345 (47) | 130 (52)       | 110 (44)          | 105 (43)       | 0.67    |
| CKD (eGFR <60 mL/min/1.73 m²)        | 418 (56) | 126 (51)       | 140 (57)          | 152 (62)       | 0.048   |
| Anemia                               | 365 (49) | 124 (50)       | 115 (46)          | 126 (51)       | 0.53    |
| COPD                                 | 163 (22) | 57 (23)        | 49 (20)           | 57 (23)        | 0.59    |
| Obesity                              | 247 (33) | 101 (41)       | 84 (34)           | 62 (25)        | 0.001   |
| Impaired iron status                 | 515 (69) | 172 (69)       | 164 (66)          | 179 (73)       | 0.278   |
| Impaired iron transport              | 438 (59) | 137 (55)       | 139 (56)          | 162 (66)       | 0.028   |
| Impaired iron storage                | 280 (38) | 104 (42)       | 88 (36)           | 280 (38)       | 0.247   |

Treatments, n (%)

| Treatment                           | Overall  | T1 (<414 pg/mL) | T2 (414–654 pg/mL) | T3 (>654 pg/mL) | P Value |
|-------------------------------------|----------|----------------|-------------------|----------------|---------|
| ACEI or ARBs                        | 578 (78) | 205 (83)       | 196 (79)          | 177 (72)       | 0.014   |
| Beta-blockers                       | 656 (88) | 214 (86)       | 224 (90)          | 218 (89)       | 0.37    |
| Aldosterone antagonists             | 308 (42) | 110 (44)       | 101 (41)          | 97 (39)        | 0.52    |
| Digoxine                            | 96 (13)  | 34 (14)        | 38 (15)           | 24 (10)        | 0.166   |
| Statins                             | 426 (57) | 147 (59)       | 139 (56)          | 140 (57)       | 0.754   |
| Loop diuretics                      | 655 (88) | 219 (88)       | 222 (90)          | 214 (87)       | 0.684   |
| Anticoagulants                      | 356 (48) | 107 (43)       | 118 (48)          | 131 (53)       | 0.079   |
| ICD                                 | 22 (3)   | 11 (5)         | 2 (1)             | 9 (4)          | 0.046   |
| CRT                                 | 10 (1)   | 4 (2)          | 4 (2)             | 2 (1)          | 0.68    |

Serum levels

| Parameter                           | Overall  | T1 (<414 pg/mL) | T2 (414–654 pg/mL) | T3 (>654 pg/mL) | P Value |
|-------------------------------------|----------|----------------|-------------------|----------------|---------|
| Hemoglobin, g/dL                    | 12.4±1.8 | 12.5±1.8       | 12.6±1.8          | 12.5±1.9       | 0.87    |
| Ferritin, µg/L [IQR]                | 142 [73–272] | 141 [75–262]  | 132 [71–251]      | 155 [70–289]   | 0.229*  |
| TSAT, %                             | 17.8 [12.2–24.5] | 18.4 [12.8–25.5] | 19.1 [13.1–25.2] | 16.1 [11.2–23.3] | <0.001* |
| Transferrin                         | 248±47   | 243±41         | 249±46            | 251±51         | 0.116   |
| Raised sTfR, n (%)                  | 133 (25) | 38 (24)        | 38 (20)           | 57 (30)        | 0.058*  |

Continued
Table 1. Continued

| Ferritin index, [IQR] | Overall n=742 | T1 (<414 pg/mL) n=248 | T2 (414–654 pg/mL) n=248 | T3 (>654 pg/mL) n=246 | P Value |
|----------------------|---------------|------------------------|--------------------------|------------------------|---------|
| Serum iron, mg/dL [IQR] | 57 [42–79] | 56 [41–77] | 61 [43–84] | 52 [40–74] | 0.007* |
| Endogenous erythropoietin, U/L [IQR] | 15 [9–25] | 14.1 [8–22] | 15 [9–25] | 17 [9–26] | 0.267* |

Data are presented as means±SD, medians (25th–75th percentile), or numbers (with percentages), where appropriate. Anemia is defined as hemoglobin level <12 g/dL in women and <13 g/dL in men. Impaired iron transport is defined as TSAT <20%; impaired iron storage is defined as ferritin <100 μg/L; and impaired iron status is defined as ferritin <100 μg/L or TSAT <20%. Raised sTfR = soluble transferrin receptor >75th percentile. ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; ferritin index, sTfR/log[Tf]; HFmrEF, heart failure with mid-range ejection fraction (defined as LVEF 40–49%); HFpEF, heart failure with preserved ejection fraction (defined as LVEF >50%); HFrEF, heart failure with reduced ejection fraction (defined as LVEF <40%); ICD, implantable cardioverter-defibrillator; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; NYHA, New York Heart Association functional class; sTfR, soluble transferrin receptor; TFAT, transferrin saturation; T1, first tertile; T2, second tertile; T3, third tertile. *P value from nonparametric tests (Kruskal–Wallis).

Unadjusted Associations Between Norepinephrine and Serum Iron Status Biomarkers

Figure 1 displays crude (unadjusted) associations between norepinephrine levels (log transformed) and levels of each iron deficiency biomarker. There was evidence of an inverse, nonlinear association between higher norepinephrine levels and lower TSAT (P value for linear component, 0.001; P value for the nonlinear component, 0.046) and of a positive, linear association between norepinephrine levels and sTfR (P value for the linear component, 0.007). In contrast, there was no evidence of a crude association between norepinephrine levels and ferritin.

In univariate logistic regression analyses (Table 2), both a log-unit increase in norepinephrine levels as well as a norepinephrine level >90th percentile (as compared with <90th percentile) was significantly associated with higher odds of iron deficiency.

Other Factors Associated With Serum Iron Status Biomarkers in Unadjusted Analyses

Table 2 presents the baseline variables that showed statistically significant associations with abnormal iron status in bivariate logistic regression analyses. Hyponatremia, anemia, and norepinephrine levels were the strongest, statistically significant clinical predictors.

Multivariable-Adjusted Associations Between Norepinephrine and Serum Iron Status Biomarkers

In line with the main objectives of the study, multivariable logistic regression analyses adjusting for variables significantly associated with iron deficiency were performed. High levels of norepinephrine (defined as norepinephrine >90th percentile of the distribution, as compared to norepinephrine ≤90th percentile) were significantly associated with impaired iron status (odds ratio, 2.23; 95% CI, 1.14–4.41), impaired iron transport (odds ratio, 2.28; 95% CI, 1.19–4.01), increased iron demand (odds ratio, 2.23; 95% CI, 1.24–4.01), whereas it was not associated with impaired iron storage (low ferritin alone) or with presence of anemia (Table 3). Interestingly, the association between norepinephrine levels and the different biomarkers suggesting iron deficiency was independent of LVEF. We examined the interaction between LVEF categories and this association, and it was not significant (P=0.865).

To analyze the association between iron deficiency and norepinephrine, which was the main objective of the study, we performed bi- and multivariable-adjusted linear regression analyses between increasing levels of log-transformed norepinephrine and levels of several serum iron status biomarkers. Results are presented in Table 4. Nnorepinephrine levels were inversely associated with TSAT (β=−0.124; P=0.003) and were positively associated with sTfR levels (β=0.115; P=0.006). On the other hand, there was no statistically significant association of norepinephrine levels with either ferritin or hemoglobin. Multivariate models analyzing iron status were adjusted by factors associated with abnormal iron status represented in Table 2 and also body mass index, because it remained at the limit of significance in the univariate analysis (odds ratio, 1.027 [0.997–1.057]; P=0.075).

Multivariable-Adjusted Associations Between Serum Iron Status and Norepinephrine Levels

Figure 2 displays the adjusted marginal means of serum norepinephrine according to different iron deficient and anemia states, obtained using fitted general linear models.
Results were consistent with the previous analyses: Norepinephrine levels were significantly higher in patients with impaired iron status than in those with normal iron status (528 [505–551] versus 482 pg/mL [448–518]; P value, 0.038); in patients with impaired iron transport than in those with normal iron transport (537 [511–566] versus 482 pg/mL [452–513]; P value, 0.012); and in patients with increased iron demand defined as levels of sTfR > 75th percentile than in those with normal iron demand (590 [540–646] versus 518 pg/mL [492–545]; P value, 0.014).

Also, there was a progressive increase in adjusted norepinephrine marginal means in each consecutive sTfR quartile. In contrast, adjusted norepinephrine levels were not significantly different according to iron storage or anemia status (all P>0.05).

Discussion
In a sample of 742 patients with CHF, raised norepinephrine levels were associated with impaired iron status and, particularly, with those biomarkers that suggest impaired
Table 3. Bi- and Multivariate-Adjusted Associations Between High Norepinephrine Levels (＞90th Percentile) and Impaired Iron Status States

|                          | Unadjusted Models | Adjusted Models |
|--------------------------|-------------------|-----------------|
|                          | OR     | 95% CI | P Value | OR     | 95% CI | P Value |
| Impaired iron status     | 2.589  | 1.334 to 5.025 | 0.005   | 2.206  | 1.105 to 4.405 | 0.025   |
| Impaired iron transport  | 2.824  | 1.566 to 5.095 | 0.001   | 2.276  | 1.192 to 4.346 | 0.013   |
| Impaired iron storage    | 1.083  | 0.656 to 1.787 | 0.756   | 1.253  | 0.728 to 2.155 | 0.415   |
| Increased iron demand    | 2.376  | 1.339 to 4.215 | 0.003   | 2.229  | 1.239 to 4.009 | 0.007   |
| Anemia                   | 1.606  | 0.983 to 2.622 | 0.058   | 1.149  | 0.657 to 2.009 | 0.627   |

Anemia is defined as hemoglobin level <12 g/dL in women and <13 g/dL in men. Impaired iron transport is defined as TSAT <20%; impaired iron storage is defined as ferritin <100 μg/L; impaired iron status is defined as ferritin <100 μg/L or TSAT <20%; and increased iron demand is defined as soluble transferrin receptor >75th percentile. Raised norepinephrine = norepinephrine serum concentration >90th percentile. OR indicates odds ratio.

Iron transport (TSAT <20%) and increased iron demand (raised levels of sTfR). This is particularly important given that these 2 iron-deficient states have been shown to strongly correlate with worse clinical profile and prognosis in patients with CHF.20 Given the proven role of neurohormonal activation and, particularly, sympathetic activation in the pathophysiological cascade leading to, and promoting the progression of, CHF, the new data emerging from our study are particularly relevant given that they may support the hypothesis that iron deficiency might not just be a comorbidity, but could also be a key element in the pathophysiological sequence leading to, and promoting the progression of, CHF. To the best of our knowledge, this is the first clinical study suggesting a potential interplay between norepinephrine levels as a surrogate of activation of the sympathetic system and the frequent dysregulation of iron metabolism observed in patients with CHF.

It is well known that iron deficiency is common in patients with CHF, and it is associated with worse quality of life, functional capacity, and prognosis.1-6 The negative impact of iron deficiency may not only be explained by the role of iron in erythropoiesis, but may also be driven by the effects of iron in cardiomyocyte function and, particularly, in mitochondrial respiration. These effects have been confirmed in cellular models where iron deficiency in the cardiomyocyte leads to mitochondrial dysfunction, hypertrophy, impaired cardiomyocyte contractility, and ventricular dysfunction21-28 and where restoration of intracellular iron levels reversed these effects.27-29 Haddad et al showed, in an animal model of cardiac iron deficiency, that the myocardium of these animals showed impaired mitochondrial respiration at the cardiomyocyte level and a blunted response in ventricular contractility after administration of dobutamine. Interestingly, these abnormalities were reversed after administration of intravenous iron.27 At a clinical level, cohort studies conducted in healthy individuals in whom impaired iron status was associated with higher risk of developing heart failure (HF) over time may give further support to the hypothesis that iron may be involved in the pathophysiological sequence leading to HF.30

Disturbances in energy metabolism at the cellular level and impairment of the contractile function of cardiomyocytes and skeletal muscle cells as a result of iron deficiency may affect patients across all LVEF categories and may promote disease progression in all of them.

A previous study of our group showed that the particular iron states that are associated with a worse clinical profile and prognosis are those that entail abnormalities in the transport of iron or an increased demand of iron at the cellular level. However, in the above-mentioned study, isolated abnormalities at the storage level, represented by normal TSAT and low ferritin, were associated with a clinical profile and prognosis undistinguishable from patients with CHF and normal iron status.20 Interestingly, in the present study, a raised sympathetic nervous system (SNS) activity, represented by raised norepinephrine levels, was particularly associated with biomarkers suggesting abnormalities in iron

Table 4. Bivariate and Multivariate-Adjusted Associations Between Increasing Levels of NE and Levels of Iron Status Biomarkers (All Log-Transformed)

| Biomarker    | Unadjusted Models | Adjusted Models |
|--------------|-------------------|-----------------|
|              | R²   | B     | P Value | R²   | B     | P Value |
| log iron     | 0.014 | -0.120 | 0.001   | 0.379 | -0.071 | 0.019   |
| log ferritin | 0.000 | -0.017 | 0.642   | 0.084 | 0.024  | 0.503   |
| log TSAT     | 0.022 | -0.148 | <0.001  | 0.255 | -0.124 | 0.003   |
| log sTfR     | 0.014 | 0.116  | 0.007   | 0.076 | 0.115  | 0.006   |
| log Hb       | 0.002 | -0.039 | 0.288   | 0.245 | 0.005  | 0.878   |

logHb indicates log haemoglobin; log NE, log norepinephrine; log sTfR, log soluble transferrin receptor; log TSAT, log transferrin saturation.
transport and increased iron demand at the cellular level. This association was independent of relevant factors such as body mass index and inflammatory status.31,32

It has been suggested that systemic iron depletion may precede iron deficiency at the myocardial level. Occult blood loss attributed to use of antiplatelet agents or anticoagulants, poor intake of iron in diet, and reduced intestinal absorption attributed to inflammation, among other factors, have been suggested as potential causes of systemic iron deficiency in these patients.33 However, despite these assumptions, the exact mechanisms by which CHF patients develop iron deficiency at a systemic and myocardial level are still unknown. In consequence, it is important to investigate alternative mechanisms that may lead to iron deficiency at the cardiomyocyte level on top of systemic iron deficiency. From this point of view, the interplay between iron metabolism disturbances and neurohormonal activation represented by raised levels of norepinephrine in patients with CHF observed in our study needs to be taken into account. Activation of the SNS, which involves an elevation of serum norepinephrine levels, has long-term deleterious effects on CHF and is one of the main therapeutic targets in HF.7,8 From our data, we suggest that the SNS could play a role in dysregulation of iron homeostasis in patients with CHF. Our observations are consistent with those from basic science models. Exposure of adult cardiomyocyte to norepinephrine leads to a downregulation of type 1 transferrin receptor expression.16 These molecular changes may lead to intracellular iron depletion. In the same study, exposure of cardiomyocytes to the beta agonist, isoprorenaline, also entails downregulation of type 1 transferrin receptor.16 In another study, Melenovsky et al analyzed cardiomyocytes from hearts of patients transplanted because of advanced HF.29 Compared with controls, cardiomyocytes of patients with HF showed reduced levels of intracellular iron. In this study, iron deficiency at the myocardial level was associated with a lower treatment rate with beta-blockers. This association may give further support to the hypothesis that the modulation of the neurohormonal drive of the sympathetic system by using beta-blockers may interplay with the development of iron abnormalities at the myocardial level in patients with HF.

Taking all into account, we may hypothesize that iron deficiency might be a relevant factor involved in the complex pathophysiological cascade of HF given that it may be involved in the initiation and progression of the functional abnormalities observed in these patients leading to clinical events and functional impairments. According to this hypothesis, iron deficiency may be not just an “unpleasant innocent bystander,” but a key factor actively involved in the mechanisms involved in onset and progression of CHF.

Study Limitations

Our study has several limitations that must be discussed. First of all, in this study, we conducted a cross-sectional analysis; therefore, causality may not be inferred. The deleterious influence between the 2 terms of the equation may be bidirectional. Raised norepinephrine levels as an expression of sympathetic activation may promote iron depletion at a cellular level, particularly in patients with systemic iron deficiency; on the other hand, it is also possible that systemic iron deficiency may lead to a greater impairment in cardiomyocyte function and mitochondrial function that would lead to more-pronounced oxidative stress, cardiomyocyte death, pump failure, and an enhanced (compensatory) neurohormonal activation represented by raised norepinephrine levels. This will promote HF progression and clinical adverse events.

Second, because this was a single-center study, our findings may not be representative of other CHF patient populations. Third, SNS activity is a complex concept that entails several biomarkers and measures. Using norepinephrine levels as a marker of sympathetic activation may
be seen as a simplification of the SNS. Third, SNS activity is a complex concept that may be defined by combined information obtained from several biomarkers and functional measures. According to this, estimation of SNS activity based on norepinephrine levels alone may be considered an oversimplification. Nevertheless, this parameter was used as a surrogate of the SNS when the neurohormonal hypothesis was developed and guided the development of key therapeutic strategies for treatment of CHF patients. Although sample processing and the laboratory method of analysis of norepinephrine are complex and may be seen as limitations of our study, we want to highlight that the procedures followed for obtaining and processing the samples and for measuring norepinephrine levels were accurate and based on standardized laboratory methods.

And, fourth, we used biomarkers that indirectly estimate the true iron status; however, these biomarkers are those used in daily clinical practice.29,34

Conclusions

In patients with CHF, an increased sympathetic activation estimated with raised norepinephrine levels is associated with impaired iron status. Specifically, we observed robust, independent associations between norepinephrine levels and dysregulation of biomarkers suggesting impaired iron transport (low TSAT) and increased iron demand (raised sTfR), whereas no associations were identified with iron storage (ferritin) or presence of anemia. Longitudinal studies are needed to better understand the directionality and potential causal relationship underlying the observed associations.

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References

1. Klip IT, Comin-Colet J, Voors AA, Ponikowski P, Enjuanes C, Banasiak W, Lok DJ, Rosentritt P, Torrens A, Polonski L, van Veldhuisen DJ, van der Meer P, Jankowska EA, Jessup M, Liede C, Nihoyannopoulos P, Parissis J, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P, ESC Scientific Document Group. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Eur Heart J. 2016;37:2192–2200.

2. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, Garcia Dieguez J, Giebisch G, Jankowska EA, Janssens O, Kober L, Komajda M, Lundqvist D, McMurray JJ, Nihoyannopoulos P, Parissis J, Pieske B, Riley JP, Rosano GMC, Rutten FH, Ruschitzka F, Rutten FH, van der Meer P, ESC Scientific Document Group. 2016 ESC guidelines for the diagnosis and treatment strategy for chronic heart failure: the Progetto Italiano per lo Studio della Funzione Sistolica nell’Insufficienza Cardiaca (PROOF) investigators. Circulation. 2016;133:1853–1943.

3. Enjuanes C, Klip IT, Brugueras J, Cladellas M, Ponikowski P, Banasiak W, van Veldhuisen DJ, van der Meer P, Jankowska EA, Comin-Colet J. Iron deficiency and health-related quality of life in chronic heart failure: results from a multicenter European study. Int J Cardiol. 2014;174:268–275.

4. Enjuanes C, Brugueras J, Grau M, Cladellas M, Gonzalez G, Merocio O, Moliner-Borja P, Verdu JM, Ferré N, Comin-Colet J. Iron status in chronic heart failure: impact on symptoms, functional class and submaximal exercise capacity. Rev Esp Cardiol (Engl Ed). 2016;69:247–255.

5. Bojarzczuk J, Jasiok K, Kastrzuka M, Kustrzycka-Kratochowl D, Nowak K, Jagielski D, Banasiak W, Jankowska EA, Ponikowski P. Iron deficiency in heart failure: impact on response to cardiac resynchronization therapy. Int J Cardiol. 2016;222:133–134.

6. Martens P, Verbrugge F, Nijst P, Dupont M, Tang WH, Mullens W. Impact of iron deficiency on response to and remodeling after cardiac resynchronization therapy. Am J Cardiol. 2017;119:65–70.

7. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, Drazner MH, Filippatos G, Fuster V, Gheorghiade M, Greenberg B, Habib S, Heidenreich PA, Hohnloser SH, Hutter AM, Jankowska EA, Jessup M, Linde C, Lutas ED, Nieminen MS, Ornato JP, Platonov P, Ponikowski P, Rehan S, Troughton RW, Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, Drazner MH, Filippatos G, Fuster V, Gheorghiade M, Greenberg B, Habib S, Heidenreich PA, Hohnloser SH, Hutter AM, Jankowska EA, Linde C, Lutas ED, Nieminen MS, Ornato JP, Platonov P, Ponikowski P, Rehan S, Troughton RW. 2016 ACC/AHA/HFSA focused update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. J Am Coll Cardiol. 2017;70:776–803.

8. Ponikowski P, Verbrugge F, Nijst P, Dupont M, Tang WH, Mullens W. Impact of iron deficiency on response to and remodeling after cardiac resynchronization therapy. Am J Cardiol. 2017;119:65–70.

9. Anker SD, Comin-Colet J, Filippatos G, Willenheimer R, Dickstein K, Drexler H, Lüscher TF, Bart B, Banasiak W, Niegowska J, Kirwan BA, Mori C, van Eisenhart Rothe B, Pocock SJ, Poole-Wilson PA, Ponikowski P; FAIR-HF Trial Investigators. Ferric carboxymaltose in patients with heart failure and iron deficiency. N Engl J Med. 2009;361:2436–2448.

10. Ponikowski P, van Veldhuisen DJ, Comin-Colet J, Erti G, Komajda M, Mareev V, McDonagh T, Parkhomenko A, Tavazzi L, Levesque V, Mori C, Roubert B, Filippatos G, Ruschitzka F, Anker SD; CONIFER-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;36:657–668.

11. Hjalmarson A, Goldstein S, Fagerberg B, Wedel H, Waagstein F, Kjekshus J, Wikstrand J, Elilall D, Vitocek J, Adlerich J, Halinen M, Dietz R, Neuhaus KL, Jänsis A, Thorgeirsson G, Dunsheim PH, Gullestad L, Kuch J, Herlitz J, Rickenbacher P, Ball S, Gottlieb S, Deedwania P; MERIT-HF Study Group. Effects of controlled-release metoprolol on total mortality, hospitalizations, and wellbeing in patients with heart failure: the Metoprolol CR/XL Randomized Intervention Trial in congestive Heart Failure (MERIT-HF). JAMA. 2000;283:1295–1302.

12. Packard C, Coats AJ, Fowler MB, Katus HA, Krum H, Mohacsi P, Rouleau JL, Tendera M, Castaigne A, Roecker EB, Schultz MK, DeMets DL. Effect of carvedilol on survival in severe chronic heart failure. N Engl J Med. 2001;344:1651–1658.

13. Packard C, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, Shusterman NH. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. N Engl J Med. 1996;334:1349–1355.

14. Packard C. Effect of carvedilol on the morbidity of patients with severe chronic heart failure: results of the Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) Study. Circulation. 2002;106:2194–2199.

15. Flather MD, Shibata MC, Coats AJ, van Veldhuisen DJ, Parkhomenko A, Borbola J, Cohen-Solal A, Dumitrascu D, Ferrari R, Lechat P, Soler-Soler J, Tavazzi L, Spinarova L, Toman J, Böhm M, Anker SD, Thompson SG, Poole-Wilson PA, SENIORS Investigators. Randomized trial to determine the effect of nebivolol on mortality and cardiovascular hospital admission in elderly patients with heart failure (SENIORS). Eur Heart J. 2006;27:215–225.

16. Maeder MT, Khammy Q, dos Remedios C, Kaye DM. Myocardial and systemic iron depletion in heart failure implications for anemia accompanying heart failure. J Am Coll Cardiol. 2011;58:474–480.

17. Ferré N, Aranyó J, Enjuanes C, Verdu-Rotellet J, Ruiz S, Gonzalez-Robledo G, Merocio O, de Ramon M, Ponikowski P, Brugueras J, Comin-Colet J. Differences in neurohormonal activation partially explain the obesity paradox in patients with heart failure: the role of sympathetic activation. Int J Cardiol. 2015;181:120–126.

18. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med. 1999;130:461–470.

19. Nutritional anemias. Report of a WHO scientific group. World Health Organ Tech Rep Ser. 1968;405:3–37.
20. 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.

21. Chen C, Paw BH. Cellular and mitochondrial iron homeostasis in vertebrates. *Biochim Biophys Acta*. 2012;1823:1459–1467.

22. Anderson GJ, Vulpe CD. Mammalian iron transport. *Cell Mol Life Sci*. 2009;66:3241–3261.

23. Richardson DR, Lane DJ, Becker EM, Huang ML, Suryo Rahmanto Y, Sheftel AD, Ponka P. Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. *Proc Natl Acad Sci USA*. 2010;107:10775–10782.

24. Bayeva M, Ardehali H. Mitochondrial dysfunction and oxidative damage to sarcomeric proteins. *Curr Hypertens Rep*. 2010;12:426–432.

25. Bayeva M, Gheorghiade M, Ardehali H. Mitochondria as a therapeutic target in heart failure. *J Am Coll Cardiol*. 2013;61:218–230.

26. Naito Y, Tsujino T, Matsumoto M, Sakoda T, Ohyanagi M, Masuyama T. Adaptive response of the heart to long-term anemia induced by iron deficiency. *Am J Physiol Heart Circ Physiol*. 2009;296:H585–H593.

27. Haddad S, Wang Y, Galy B, Korf-Klingebiel M, Hirsch V, Baru AM, Rostami F, Rebull MR, Heineke J, Fliegel U, Groos S, Renner A, Toischer K, Zimmermann F, Engel S, Jordan J, Bauersachs J, Hentze MW, Willett KC, Kempf T. Iron-regulatory proteins secure iron availability in cardiomyocytes to prevent heart failure. *Eur Heart J*. 2017;38:362–372.

28. Hoes MF, Grote Beverborg N, Klijstra JD, Kuipers J, Swinkels DW, Giepmans BN, Rodenburg RJ, van Veldhuisen DJ, de Boer RA, van der Meer P. Iron deficiency impairs contractility of human cardiomyocytes through decreased mitochondrial function. *Eur J Heart Fail*. 2018;20:910–919.

29. Melenovsky V, Petrak J, Mracek T, Stenes J, Borlau BA, Nuskova H, Pluhacek T, Spatenka J, Kovalskova J, Drahota Z, Kautner J, Pirk J, Houstek J. Myocardial iron content and mitochondrial function in human heart failure: a direct tissue analysis. *Eur J Heart Fail*. 2017;19:522–530.

30. Silvestre OM, Gonçalves A, Nadruz W Jr, Claggett B, Couper D, Eckfeldt JH, Pankow JS, Anker SD, Solomon SD. Ferritin levels and risk of heart failure—the Atherosclerosis Risk in Communities Study. *Eur J Heart Fail*. 2017;19:340–347.

31. Tkaczyzyn M, Drozd M, Wegrzynowska-Teodorczyk K, Flinta I, Kobak K, Banasiak W, Ponikowski P, Jankowska EA. Depleted iron stores are associated with inspiratory muscle weakness independently of skeletal muscle mass in men with systolic chronic heart failure. *J Cachexia Sarcopenia Muscle*. 2018;9:547–556.

32. Van Aelst LNL, Abraham M, Sadoune M, Lefebvre T, Manivet P, Logeart D, Launay JM, Karim Z, Puy H, Cohen-Solal A. Iron status and inflammatory biomarkers in patients with acutely decompensated heart failure: early in-hospital phase and 30-day follow-up. *Eur J Heart Fail*. 2017;19:1075–1076.

33. Jankowska EA, Rozentryt P, Witkowska A, Nowak J, Hartmann O, Ponikowski B, Borodulin-Nadzieja L, Banasiak W, Polonski L, Filippatos G, McMurray JJ, Anker SD, Ponikowski P. Iron deficiency: an ominous sign in patients with systolic chronic heart failure. *Eur Heart J*. 2010;31:1872–1880.

34. Grote Beverborg N, Klip IT, Meijers WC, Voors AA, Veger EL, van der Wal HH, Swinkels DW, van Pelt J, Mulder AB, Bulstra SK, Vellenga E, Mariani MA, de Boer RA, van Veldhuisen DJ, van der Meer P. Definition of iron deficiency based on the gold standard of bone marrow iron staining in heart failure patients. *Circ Heart Fail*. 2018;11:e004519.