ORIGINAL RESEARCH

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

Pedro Moliner, MD; Cristina Enjuanes, MD, PhD; Marta Tajes, PhD; Miguel Cainzos-Achirica, MD, MPH; Josep Lupón, MD, PhD; Alberto Garay, MD, PhD; Santiago Jimenez-Marrero, MD; Sergi Yun, MD; Núria Farré, MD, PhD; Mercé Cladellas, MD, PhD; Carles Díez, MD; Jose Gonzalez-Costello, MD; Josep Comin-Colet, MD, PhD

***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

From the Heart Failure Unit, Department of Cardiology, Hospital Universitari Germans Trias i Pujol, Badalona, Spain (P.M., J.L.); Department of Medicine, Universitat Autònoma de Barcelona, Spain (P.M., J.L., N.F., M.C.); Community Heart Failure Program, Cardiology Department, Bellvitge University Hospital (C.E., M.C.-A., A.G., S.J.-M., S.Y., J.C.-C.), Cardiovascular Diseases Research Group, Bellvitge Biomedical Research Institute (IDIBELL; C.E., M.C.-A., A.G., S.J.-M., S.Y., C.D., J.G.-C., J.C.-C.), and Advanced heart Failure and Heart Transplant Unit, Department of Cardiology, Bellvitge University Hospital (C.D., J.G.-C.), Hospitalet de Llobregat, Barcelona, Spain; Heart Diseases Biomedical Research Group, Program of Research in Inflammatory and Cardiovascular Disorders, Hospital del Mar Biomedical Research Institute (IMIM), Barcelona, Spain (C.E., M.T., N.F., J.C.-C.); Johns Hopkins Ciccarone Center for the Prevention of Heart Disease, Department of Cardiology, Johns Hopkins Medical Institutions, Baltimore, MD (M.C.-A.); School of Medicine and Medical Sciences, Universitat Internacional de Catalunya, Sant Cugat del Valles, Barcelona, Spain (M.C.-A.); Heart Failure Program, Department of Cardiology, Hospital del Mar, Parc de Salut Mar, Barcelona, Spain (N.F.); Department of Cardiology, Hospital del Mar, Parc de Salut Mar, Barcelona, Spain (M.C.); Department of Clinical Sciences, School of Medicine, Universitat de Barcelona, Spain (J.C.-C.).

Correspondence to: Josep Comin-Colet, MD, PhD, Hospital Universitari de Bellvitge, Department of Cardiology, 19th Floor, Feixa Llarga s/n, 08907 Hospitalet de Llobregat, Barcelona, Spain. E-mail: jcomin@bellvitgehospital.cat

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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 raised 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,[7,8,11–15] 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.[16] 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.[2,17] 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.[17] 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) $\times$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.[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 >75th 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).[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 χ^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 >90th percentile [1050 pg/mL] of the distribution in the study population, as compared with ≤90th 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.[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

	Overall n=742	T1 (<414 pg/mL) n=248	T2 (414–654 pg/mL) n=248	T3 (>654 pg/mL) n=246	*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/m^2	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 m^2	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
HFmrEF, n (%)	116 (16)	45 (18)	38 (15)	33 (13)	0.346
HFrEF, 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 (%)					
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 m^2)	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 (%)					
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					
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

	Overall n=742	T1 (<414 pg/mL) n=248	T2 (414–654 pg/mL) n=248	T3 (>654 pg/mL) n=246	*P* Value
Ferritin index, [IQR]	0.76 [0.54–1.14]	0.72 [0.53–1.14]	0.72 [0.55–1.06]	0.8 [0.56–1.20]	0.348*
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 resincronization therapy; ferritin index, sTfR/log10[ferritin]; 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; TSAT, 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, 2.21; 95% CI, 1.11–4.41), impaired iron transport (odds ratio, 2.28; 95% CI, 1.19–4.35), and 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.

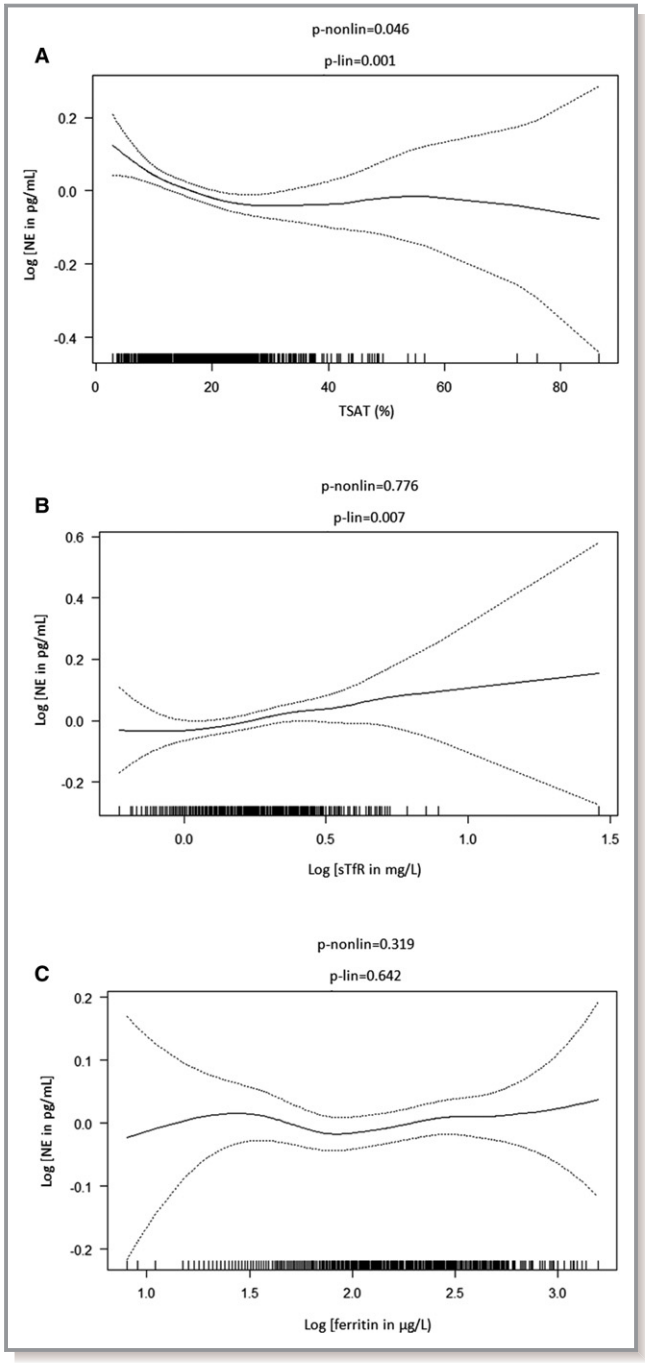

Figure 1. Unadjusted associations between norepinephrine levels (log-transformed) and levels of serum biomarkers of iron status. The associations were calculated using generalized additive models (GAM). Association between log norepinephrine serum levels and log TSAT **(A)**, log NE serum levels and log sTfR **(B)**, and log norepinephrine serum levels and log ferritin **(C)**. sTfR indicates soluble transferrin receptor; TSAT, transferrin saturation.

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).

Table 2. Baseline Variables With Statistically Significant Associations With Abnormal Iron Status in Bivariate Logistic Regression Analyses

	OR (95% CI)	*P* Value
Age (per y)	1.026 (1.012–1.040)	<0.001
Sex (male vs female)	1.654 (1.198–2.284)	0.002
DM (yes vs no)	1.533 (1.116–2.105)	0.008
SBP (per mm Hg)	1.008 (1.001–1.015)	0.021
LVEF (per 1%)	1.012 (1.003–1.021)	0.012
eGFR (per 1 mL/min/1.73 cm^2)	0.991 (0.985–0.997)	0.006
logNT-proBNP (per 1 pg/mL)	1.553 (1.187–2.031)	0.001
Albumin (per 1 g/dL)	0.555 (0.388–0.792)	0.001
hs-CRP (per 1 mg/L)	1.183 (1.076–1.301)	0.001
Hemoglobin (per 1 g/dL)	0.727 (0.662–0.797)	<0.001
Anemia (yes vs no)	2.707 (1.912–3.833)	<0.001
ACEI (yes vs no)	0.486 (0.345–0.684)	<0.001
ARB (yes vs no)	2.066 (1.322–3.229)	0.001
HDZ+NTG (yes vs no)	2.060 (1.335–3.181)	0.001
MRA (yes vs no)	0.718 (0.524–0.984)	0.039
Na <135 (yes vs no)	2.844 (1.091–7.411)	0.032
NYHA III to IV (yes vs no)	1.438 (1.045–1.977)	0.026
logNE (per log 1 pg/mL)	2.026 (1.091–3.762)	0.025
NE>p90 (yes vs no)	2.737 (1.414–5.299)	0.003

Anemia is defined as hemoglobin level <12 g/dL in women and <13 g/dL in men. Impaired iron status is defined as ferritin <100 µg/L or TSAT <20%. ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HDZ+NTG, hydralazine and nitrates; hs-CRP, high-sensitivity C-reactive protein; logBNP, log N-terminal pro-brain-type natriuretic peptide; logNE, log norepinephrine concentration; LVEF, left ventricular ejection fraction; MRA, mineralcorticoid receptor antagonists; NE>p90, norepinephrine serum concentration >90th percentile (1050 pg/mL); NYHA, New York Heart Association functional class; SBP, systolic blood pressure.

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

	Raised Norepinephrine					
	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 cardiomyocite contractility, and ventricular dysfunction[21–28] 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)

	log NE					
	Unadjusted Models			Adjusted Models		
	R^2	B	*P* Value	R^2	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; logsTfR, log soluble transferrin receptor; logTSAT, log transferrin saturation.

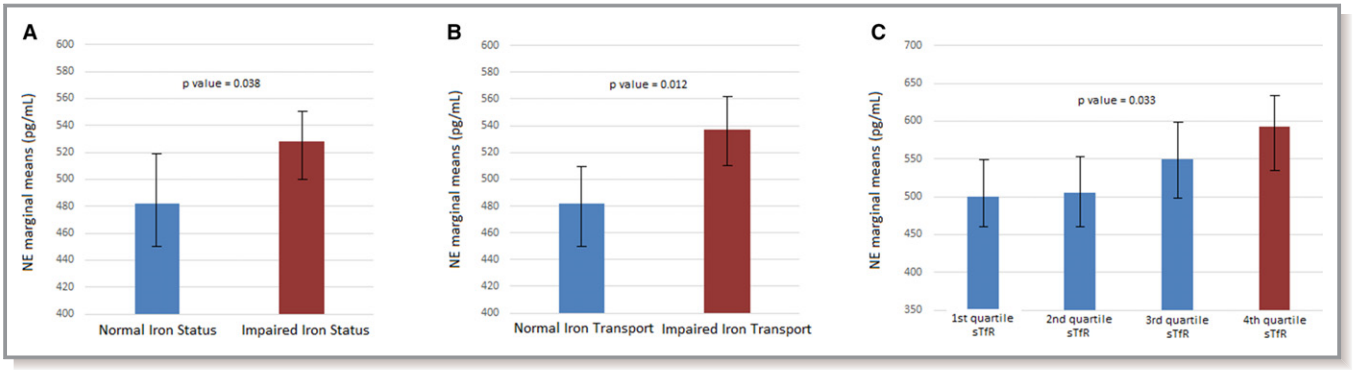

Figure 2. Predicted adjusted marginal means of serum norepinephrine levels by iron status categories. Norepinephrine adjusted marginal means calculated with general linear models according to **(A)** iron status, **(B)** iron transport, and **(C)** iron demand: sTfR classified in quartiles (Q1 ≤1.21 mg/L; Q2 1.22–1.60 mg/L; Q3 1.61–2.24 mg/L; Q4 ≥2.25 mg/L). Impaired iron status was defined as TSAT <20% or ferritin <100 ng/mL. Impaired iron transport was defined as TSAT <20%. sTfR indicates soluble transferrin receptor; TSAT, 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, isoprenaline, 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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Disclosures

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