This study is a post-hoc analysis of the ‘systems Biology Study to Tailored Treatment in Chronic Heart Failure’ (BIOSAT-CHF), which has been described previously. In short, BIOSAT-CHF prospectively enrolled 2516 patients with worsening signs and/or symptoms of HF and a left ventricular ejection fraction ≤40% or brain natriuretic peptide levels >400 pg/mL or N-terminal prohormone of brain natriuretic peptide >2000 pg/mL. BIOSAT-CHF was approved by local medical ethics committees at each participating centre. We measured the following biomarkers in 2279 stored samples, drawn at the time of presentation at the emergency department or hospital admission: iron, transferrin saturation (TSAT), ferritin, hepcidin, soluble transferrin receptor (sTfR) and FGF23 [using a c-terminal ELISA (ImmunoTech, Inc., San Clemente, CA, USA), measuring both intact and c-terminal FGF23 cumulatively]. Univariable and multivariable linear regression analyses were performed using log-transformed FGF23 levels as dependent variables and log-transformed TSAT, sTfR, ferritin and hepcidin as independent variables. The multivariable models were adjusted for predictors that have previously been associated with FGF23. Univariable and FGF23-adjusted restricted cubic splines based on Cox proportional hazard regression were constructed to assess the association between iron parameters and all-cause mortality. Mediation analyses were performed according to the methods described by Baron and Kenny.

Baseline characteristics of all patients are depicted in Table 1. Mean (± standard deviation) age of the patients was 69 ± 12 years, 26.1% were female and median (interquartile range) left ventricular ejection fraction was 30% (25–36%). Patients with higher FGF23 levels were more frequently female, had lower ferritin, TSAT, and hepcidin levels and higher levels of inflammatory markers (P for trend <0.001). FGF23 levels were strongly correlated to TSAT (Spearman’s ρ = –0.42), sTfR (ρ = 0.43), ferritin (ρ = –0.31) and hepcidin (ρ = –0.37; all P < 0.0001). Individual levels of TSAT, sTfR, ferritin and hepcidin were the strongest predictors of FGF23 levels compared to previously established determinants of FGF23 levels (all P < 0.001). During a median follow-up of 21 months (interquartile range 16–27 months), 596 patients (26%) died. Continuous iron parameter levels were strongly associated with prognosis in univariable analyses (all P < 0.001) (Figure 1). When adjusting for FGF23, all iron parameters lost their predictive value. There was a significant interaction between TSAT and FGF23 on outcome (P = 0.012). Moreover, we identified a highly significant interaction between a history of renal disease and FGF23 in the association between iron parameters and all-cause mortality (P < 0.01). Finally, we evaluated whether the association between iron parameters and all-cause mortality was mediated by FGF23. FGF23 levels significantly mediated the association between TSAT, ferritin, sTfR, and hepcidin and all-cause mortality [P for indirect effect (FGF23-mediated) <0.0001]. The direct effect (non-FGF23-mediated) was not significant for all iron parameters in these models (Table 1). As a sensitivity analysis, we also evaluated whether inflammation alters the association between iron status and outcome. Adjustment for C-reactive protein and interleukin-6 did not affect the prognostic consequences of iron parameters.

In this study, we found that in a large, multinational cohort of worsening HF patients, iron parameters are independently related to FGF23 levels. Second, the prognostic value of iron parameters is significantly mediated by FGF23. Taken these findings together, our data provide insight into the pathophysiology of ID in HF patients, in which FGF23 may play a prominent role.

Low iron has previously been identified as one of the drivers of FGF23 production in vivo. By stabilizing hypoxia-inducible factor 1-α, ID up-regulates the expression of the proprotein convertase furin, which in turn increases FGF23 production and cleavage into intact and c-terminal FGF23. Observational studies in chronic kidney disease and general population cohorts identified an association between ID and outcome, which was mediated by FGF23. For example, an observational study in renal transplant recipients yielded comparable results to our study, showing that the association between ID and outcome is considerably mediated by FGF23 levels and not by inflammation. Furthermore, FGF23 was strongly linked to...
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Mediation analysis was performed for transferrin saturation, sTfR, ferritin and hepcidin. The direct effect is the effect of the respective iron status parameter on all-cause mortality after correcting for interleukin-6; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro B-type natriuretic peptide; sTfR, soluble transferrin receptor.

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; FGF23, fibroblast growth factor 23; IL-6, interleukin-6; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro B-type natriuretic peptide; sTfR, soluble transferrin receptor.

Table 1 Baseline characteristics, stratified by fibroblast growth factor 23 levels in tertiles, and multivariable mediation analysis of iron status parameters through fibroblast growth factor 23

| Demographics | Total cohort (n = 2297) | FGF23 ≤ 140 RU/mL (n = 766) | FGF23 141–386 RU/mL (n = 766) | FGF23 ≥ 387 RU/mL (n = 765) | P-value for trend | Mediation analysisa |
|--------------|------------------------|-----------------------------|-------------------------------|-----------------------------|-----------------|---------------------|
|              |                        |                             |                               |                             |                 |                     |
| Age (years)  | 69 ± 12                | 66 ± 11                     | 70 ± 12                       | 70 ± 12                     | <0.001 |                     |
| Women        | 600 (26.1)             | 162 (21.1)                  | 201 (26.2)                    | 237 (31.0)                  | <0.001 |                     |
| BMI (kg/m²)  | 27.9 ± 5.4             | 28.0 ± 4.9                  | 27.7 ± 5.7                    | 28.0 ± 5.7                  | 0.381  |                     |
| LVEF (%)     | 30 (25–36)             | 30 (25–35)                  | 30 (25–37)                    | 30 (23–37)                  | 0.247  |                     |
| HF septology | 1045 (46.3)            | 345 (45.6)                  | 339 (45.4)                    | 361 (47.9)                  | 0.369  |                     |
| Ischaemic    | 232 (10.3)             | 77 (10.2)                   | 89 (11.9)                     | 66 (8.8)                    | 0.13   |                     |
| Hypertension | 169 (7.5)              | 23 (3.0)                    | 63 (8.5)                      | 83 (11.0)                   | <0.001 |                     |
| Valvar disease | 130 (5.8)              | 29 (3.8)                    | 55 (7.4)                      | 46 (6.1)                    | 0.012  |                     |
| Other        | 113 (5.0)              | 41 (5.4)                    | 27 (3.6)                      | 45 (6.0)                    | 0.094  |                     |
| Estimated protein intake (g/day) | 55 ± 11 | 58 ± 12 | 55 ± 11 | 52 ± 9 | <0.001 |                     |
| KCCQ score   | 50 ± 22                | 59 ± 21                     | 49 ± 21                       | 40 ± 21                     | <0.001 |                     |
| Laboratory   |                        |                             |                               |                             |       |                     |
| Iron (mg/dL) | 45 (28–73)             | 62 (39–84)                  | 45 (28–73)                    | 34 (22–56)                  | <0.001 |                     |
| Transferrin (mg/dL) | 200 (160–250) | 200 (170–240) | 200 (160–250) | 210 (160–260) | 0.006 |                     |
| Iron deficiency a | 1413 (61.5) | 323 (42.2) | 474 (61.9) | 616 (80.5) | <0.001 |                     |
| Haptoglobin (mmol/L) | 6.3 (2.2–16.4) | 9.0 (5.0–20.7) | 7.5 (3.1–18.6) | 2.3 (0.8–8.4) | <0.001 |                     |
| sTfR (mg/L)  | 1.5 (1.2–2.1)          | 1.3 (1.0–1.6)               | 1.5 (1.2–2.0)                 | 2.0 (1.4–2.8)               | <0.001 |                     |
| Transferrin saturation (%) | 17 (11–25) | 22 (16–29) | 17 (12–24) | 12 (8–18) | <0.001 |                     |
| Ferritin (μg/L) | 102 (50–193) | 143 (83–240) | 103 (58–190) | 63 (31–139) | <0.001 |                     |
| IL-6 (pg/mL) | 5.1 (2.8–10.2)         | 3.0 (1.8–5.6)               | 5.1 (3.0–8.9)                 | 8.6 (5.0–16.1)              | <0.001 |                     |
| CRP (mg/L)   | 13 (6–26)              | 8 (4–19)                    | 14 (6–26)                     | 18 (9–32)                   | 0.001  |                     |
| Haemoglobin (g/dL) | 132 ± 1.9 | 138 ± 1.6 | 133 ± 1.9 | 126 ± 1.9 | <0.001 |                     |
| Anaemia      | 753 (36.0)             | 136 (20.5)                  | 252 (35.6)                    | 365 (50.3)                  | <0.001 |                     |
| NT-proBNP (ng/L) | 2680 (1173–5696) | 1302 (511–2811) | 2913 (1429–5357) | 4688 (2408–9852) | <0.001 |                     |
| Medication   |                        |                             |                               |                             |       |                     |
| Beta-blockers | 1912 (83.2)            | 666 (86.9)                  | 637 (83.2)                    | 609 (79.6)                  | <0.001 |                     |
| (on target dose) | 125 (5.4) | 31 (4.0) | 41 (5.4) | 53 (6.9) | 0.013  |                     |
| ACEi/ARBs    | 1662 (72.4)            | 601 (78.5)                  | 565 (73.8)                    | 496 (64.8)                  | <0.001 |                     |
| ACEi/ARBs (on target dose) | 300 (13.1) | 133 (17.4) | 99 (12.9) | 68 (8.9) | <0.001 |                     |
| Loop diuretics | 2287 (99.6) | 760 (99.2) | 763 (99.6) | 764 (99.9) | 0.053  |                     |
| Aldosterone antagonists | 1235 (53.8) | 421 (55.0) | 403 (52.6) | 411 (53.7) | 0.628  |                     |
| Proton pump inhibitors | 806 (35.1) | 213 (27.8) | 269 (35.1) | 324 (42.4) | <0.001 |                     |
| P2Y12 inhibitors | 356 (15.5) | 137 (17.9) | 118 (15.4) | 101 (13.2) | 0.011  |                     |
| Oral anticoagulants | 881 (38.4) | 225 (29.4) | 333 (43.5) | 323 (42.2) | <0.001 |                     |

Values are given as mean ± standard deviation, n (%), or median (interquartile range).
ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; FGF23, fibroblast growth factor 23; IL-6, interleukin-6; KCCQ, Kansas City Cardiomyopathy Questionnaire; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro B-type natriuretic peptide; sTfR, soluble transferrin receptor.

aMediation analysis was performed for transferrin saturation, sTfR, ferritin and hepcidin. The direct effect is the effect of the respective iron status parameter on all-cause mortality after correcting for FGF23; the indirect effect is the FGF23-mediated effect of the respective iron status parameter on all-cause mortality. The total effect of iron status on mortality is a composite of the indirect (FGF23-related) and direct (non-FGF23-related) pathways. CIs are bootstrapped 2000 times. The mediation model is corrected for age, haemoglobin, NT-proBNP, serum urea, and the use of beta-blockers at baseline.

Iron deficiency was defined as transferrin saturation <20%.
Figure 1 Association between iron status parameters (transferrin saturation, ferritin and soluble transferrin receptor) and all-cause mortality, before and after correction for fibroblast growth factor 23 (FGF23). Data were fit by Cox proportional hazard regression models using restricted cubic splines. CI, confidence interval; HR, hazard ratio.

iron parameters in these patients, similar to our findings.

Given the strong link between iron status and FGF23 levels, one may expect changes in FGF23 levels after iron administration. Conflicting data have been published on the effect of intravenous iron administration on FGF23 levels. In a small cohort of iron-deficient patients with HF and reduced ejection fraction, c-terminal FGF23 levels decreased after ferric carboxymaltose (FCM) administration during 28 days of follow-up, with transient hypophosphataemia and short-term increase in intact FGF23 levels.9 Contrarily, another study comparing FCM and iron sucrose in haemodialysis patients found a short-term drop in intact FGF23 and a rise in c-terminal FGF23 in patients receiving FCM, whereas iron sucrose did not affect these parameters.10 Currently, no large studies on the effect of different iron agents on FGF23 levels in HF have been published. When intravenous iron therapy substantially and persistently lowers FGF23 levels, this might provide an additional explanation for the mode of action of this treatment modality, besides its beneficial haematological effects.

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Our study has several strengths and limitations. First, we used a large and well-described cohort of worsening HF patients, in which we measured FGF23 and multiple iron parameters. We acknowledge the observational nature of our study, making it challenging to directly study pathophysiological mechanisms. Second, using the FGF23 assay we used for our study, we could not distinguish c-terminal FGF23 from intact FGF23. Finally, most patients in BIOSTAT-CHF have a reduced ejection fraction. Our data should therefore be cautiously interpreted in HF patients with a preserved ejection fraction.

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