Cardiac iron concentration in relation to systemic iron status and disease severity in non-ischaemic heart failure with reduced ejection fraction

Valentin G. Hirsch1,2, Jörn Tongers2, Julia Bode3, Dominik Berliner2, Julian D. Widder2, Felicitas Escher4, Vitalii Mutsenko5, Bomee Chung1,2, Fatemeh Rostami1,2, Anja Guba-Quint1,2, Evangelos Giannitsis6, Heinz-Peter Schultheiss4, Carla Vogt3, Johann Bauersachs2, Kai C. Wollert1,2, and Tibor Kempf1,2*

1Division of Molecular and Translational Cardiology, Hannover Medical School, Hannover, Germany; 2Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany; 3Institute for Analytical Chemistry, University of Mining and Technology, Freiberg, Germany; 4Institute for Cardiac Diagnostics and Therapy, Berlin, Germany; 5Institute for Multiphase Processes, Leibniz University, Hannover, Germany; and 6Cardiology, Department of Internal Medicine III, University Hospital Heidelberg, Heidelberg, Germany

Received 24 May 2019; revised 11 February 2020; accepted 12 February 2020; online publish-ahead-of-print 10 March 2020

Aims
Low cardiac iron levels promote heart failure in experimental models. While cardiac iron concentration (CI) is decreased in patients with advanced heart failure with reduced ejection fraction (HFrEF), CI has never been measured in non-advanced HFrEF. We measured CI in left ventricular (LV) endomyocardial biopsies (EMB) from patients with non-advanced HFrEF and explored CI association with systemic iron status and disease severity.

Methods and results
We enrolled 80 consecutive patients with non-ischaemic HFrEF with New York Heart Association class II or III symptoms and a median (interquartile range) LV ejection fraction of 25 (18–33)%. CI was 304 (262–373) μg/g dry tissue. CI was not related to immunohistological findings or the presence of cardiotropic viral genomes in EMBs and was not related to biomarkers of systemic iron status or anaemia. Patients with CI in the lowest quartile (CIQ1) had lower body mass indices and more often presented with heart failure histories longer than 6 months than patients in the upper three quartiles (CIQ2–4). CIQ1 patients had higher serum N-terminal pro-B-type natriuretic peptide levels than CIQ2–4 patients [3566 (1513–6412) vs. 1542 (526–2811) ng/L; P = 0.005]. CIQ1 patients also had greater LV end-diastolic (P = 0.001) and end-systolic diameter indices (P = 0.003) and higher LV end-diastolic pressures (P = 0.046) than CIQ2–4 patients.

Conclusion
Low CI is associated with greater disease severity in patients with non-advanced non-ischaemic HFrEF. CI is unrelated to systemic iron homeostasis. The prognostic and therapeutic implications of CI measurements in EMBs should be further explored.

Keywords
Iron deficiency • Non-ischaemic heart failure with reduced ejection fraction • Endomyocardial biopsy • Inductively-coupled plasma optical emission spectroscopy

*Corresponding author. Klinik für Kardiologie und Angiologie, Medizinische Hochschule Hannover, Carl-Neuberg-Straße 1, 30625 Hannover, Germany. Tel: +49 511 532-2229, Fax: +49 511 532-3357, Email: kempf.tibor@mh-hannover.de

© 2020 The Authors. European Journal of Heart Failure published by John Wiley & Sons Ltd on behalf of European Society of Cardiology. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
Introduction

Systemic iron deficiency (ID) is a frequent comorbidity in heart failure (HF). Based on serum markers of depleted body iron stores, reduced systemic iron availability, and unmet cellular iron requirements, ~50% of patients with HF with reduced ejection fraction (HFrEF) are iron-deficient.5-7 Systemic ID is associated with reduced exercise tolerance, increased symptom severity, and higher mortality rates independent of coexisting anemia.4-11 Iron supplementation using intravenous ferric carboxymaltose has been shown to improve exercise capacity and symptoms and to reduce the number of HF hospitalisations in iron-deficient patients with HFrEF.12-14 Current European Society of Cardiology (ESC) guidelines therefore recommend assessing systemic iron status in patients with symptomatic HFrEF.15

Iron is an essential cofactor in haem and iron–sulphur cluster-containing proteins required for oxygen transport (haemoglobin) and storage (myoglobin) as well as cellular energy metabolism (e.g. components of the mitochondrial electron transport chain).16 Using gene-targeted mice with cardiomyocyte-selective ID, we have recently observed that a ~30% decrease in cardiac iron content impairs cardiac contractile reserve and promotes adverse left ventricular (LV) remodelling after myocardial infarction.17 Transgenic mice engineered to develop even more pronounced reductions in cardiac iron spontaneously develop HF and die prematurely.18,19 Collectively, these studies indicate that a low cardiac iron content promotes HF independent of systemic iron status.

Cardiac iron concentration (CI) is 15–32 lower in patients with advanced HF undergoing heart transplantation compared to non-transplanted donor hearts.17,20-22 Notably, CI has never been measured in non-advanced HF, and it is not known if CI is related to systemic iron status in these patients. We therefore measured CI in LV endomyocardial biopsies (EMBs) from patients with non-advanced non-ischaemic HFrEF and explored CI relationship to systemic iron status and disease severity.

Methods

Study population

We studied 80 consecutive patients older than 18 years with New York Heart Association (NYHA) class II or III symptoms and LV ejection fraction (LVEF) ≤40% referred to the Department of Cardiology and Angiology at Hannover Medical School between May 2017 and June 2018 for diagnostic work-up of HFrEF with unknown aetiology. According to the updated Heart Failure Association-ESC criteria for defining advanced HF,23 all patients had non-advanced HF. All patients underwent transthoracic echocardiography and coronary angiography.

One day before coronary angiography, patients were informed about the study, which included performing an LV EMB in case coronary artery disease was excluded (no >50% diameter stenosis in a major coronary artery). Biopsies were not strictly indicated in some patients,24-25 and we discussed this beforehand with the Ethics Committee of Hannover Medical School in light of previous studies indicating the safety of the procedure.26-28 We obtained ethical approval (no. 7408–2017), and all patients provided written informed consent. LV EMBs were not associated with any complications in our patients.
immunoreactive peptide (NT-proBNP) are described in online supplementary Table S1. Following current ESC guidelines,\textsuperscript{13} diagnosis of systemic ID required a serum ferritin concentration $<100 \mu g/L$ or a serum ferritin concentration between 100 and $299 \mu g/L$ in combination with a TSAT $<20\%$. Anaemia was diagnosed using World Health Organisation haemoglobin thresholds ($<13 g/dL$ in men, $<12 g/dL$ in women). Estimated glomerular filtration rate (GFR) was calculated by the Chronic Kidney Disease-Epidemiology Collaboration equation.\textsuperscript{20} CI in EMBs was measured by inductively-coupled plasma optical emission spectroscopy (ICP-OES; a detailed description is provided in online supplementary Methods S1). To enable a comparison of our results with previous studies that have measured CI in advanced HF using inductively-coupled plasma mass spectrometry (ICP-MS), we established the ICP-MS method as previously described\textsuperscript{22} and measured iron concentration in five EMBs with ICP-OES and ICP-MS. Iron concentrations determined with both methods were almost identical [289 (242–406) vs. 291 (246–416) $\mu g/g$ dry tissue].

### Immunohistological and virological analyses

Cardiomyocyte diameters were determined in haematoxylin- and eosin-stained sections. For immunohistological evaluation, specimens were embedded in Tissue-Tek O.C.T. compound (Sakura), snap-frozen in methylenebutane that had been cooled in liquid nitrogen, and stored at $-80^\circ C$ until processing. Serial 5 $\mu m$ cryosections were placed on 10% poly-L-lysine-precoated slides. Type I and type III collagens were detected with antibodies from Biotrend and Calbiochem, respectively. Sections were also stained with CD3 (Dako), CD45RO (Dako), LFA-1 (ImmunoTools), MAC-1 (ImmunoTools), and perforin (BD Biosciences) antibodies and EnVision peroxidase-conjugated secondary antibodies (Dako). 3-Amino-9-ethylcarbazole (Merck) was used as chromogenic substrate. Slides were then counterstained with haematoxylin and mounted in Aquatex (Merck). Inflammatory cells were quantified at 200-fold magnification by digital image analysis applying colour-coded thresholds.\textsuperscript{31} Active myocarditis was defined by the presence of $\geq 14$ leucocytes/mm$^2$, including up to 4 monocytes/mm$^2$ with the presence of $\geq 7$ CD3$^+$ T-lymphocytes/mm$^2$, which are focally associated with cardiomyocyte degeneration and necrosis.\textsuperscript{25} Non-ischaemic cardiomyopathy with high-grade inflammation was defined by the presence of $\geq 14$ leucocytes/mm$^2$, including up to 4 monocytes/mm$^2$ with the presence of $\geq 7$ CD3$^+$ T-lymphocytes/mm$^2$, without cardiomyocyte degeneration and necrosis. Cardiotropic viral genomes were detected by nested polymerase chain reaction on RNA (enterovirus); DNA (adenovirus, Epstein–Barr virus, human herpesvirus 6); or RNA and DNA (parvovirus B19).\textsuperscript{32}

### Statistical analysis

Categorical variables are reported as numbers and percentages, continuous variables as median with interquartile range (IQR). Proportions were compared by the chi-square test, continuous variables by the Mann–Whitney test. We used ANOVA for comparisons among more than two groups followed by Dunn’s post hoc test for comparisons between two groups. Binary logistic regression analyses were applied to identify factors that were independently associated with low CI or NT-proBNP. Spearman’s correlation analysis was applied to assess the relationship between two variables. Linear regression analysis was used to identify factors associated with systemic iron parameters and CI. A two-tailed $P$-value of $<0.05$ was considered to indicate statistical significance. Analyses were performed with GraphPad Prism 7.04 (GraphPad Software) and SPSS Statistics 25 (IBM).

### Results

#### Patients

The patient population included 53 men and 27 women. Patients had a median (IQR) age of 61 (51–71) years, a LVEF of 25 (18–33)$\%$, and NT-proBNP concentrations of 1859 (698–3740) ng/L. Patients presented with NYHA class II (64%) or class III symptoms (36%). Median HF duration was 2 (1–6) months.

#### Systemic iron status

Overall, 46% of the patients (37 out of 80) had systemic ID. Patient characteristics according to systemic iron status are shown in online supplementary Table S2. Systemic ID was more frequent in women and patients with HF histories longer than 6 months. Patients with systemic ID had lower haemoglobin concentrations and were more often anaemic. Additionally, patients with systemic ID had lower serum concentrations of ferritin and iron, lower TSATs, lower plasma hepcidin concentrations, and higher serum concentrations of sTFR and IL-6. Estimated GFR and serum concentrations of CRP, GDF-15, and NT-proBNP were not significantly different between patients with or without systemic ID (online supplementary Table S2).

Lower serum iron concentration and a higher ferritin concentration were related to higher serum concentrations of CRP and IL-6. TSAT was also inversely related to the IL-6 concentration. Higher sTFR level and a lower haemoglobin concentration were related to a higher GDF-15 concentration (online supplementary Figure S2).

#### Cardiac iron concentration

Median CI was 304 (IQR 262–373; range 132–1339) $\mu g/g$ dry tissue. CI was similar in patients with or without systemic ID [301 (228–382) vs. 312 (266–367) $\mu g/g$; $P = 0.44$]. A total of 60% of patients (12 out of 20) in the lowest CI quartile (CI$_{Q1}$) and 42% of patients (25 out of 60) in the top three CI quartiles (CI$_{Q2-4}$) had systemic ID ($P = 0.15$). Systemic iron homeostasis parameters (serum ferritin, serum iron, TSAT, sTFR, hepcidin) and haemoglobin concentrations were not significantly different between CI$_{Q1}$ and CI$_{Q2-4}$ patients (Table 1) and not associated with CI in linear regression analyses (online supplementary Figure S3). CI$_{Q1}$ patients had lower body mass indices (BMI) ($P < 0.001$) and more often presented with HF histories longer than 6 months ($P = 0.027$) than CI$_{Q2-4}$ patients (Table 1). NYHA class, estimated GFR and serum concentrations of CRP, IL-6, and GDF-15 were not significantly different between CI$_{Q1}$ and CI$_{Q2-4}$ patients (Table 1). A multivariate analysis established low BMI and HF duration longer than 6 months as independent predictors of low

\[ \text{BMI} \leq 20 \text{ and HF duration longer than 6 months} \]
Cardiac iron in non-ischaemic heart failure

Table 1  Patient characteristics according to cardiac iron concentration

|                         | All patients (n = 80) | Cardiac iron Q₁ (n = 20) | Cardiac iron Q₂–₄ (n = 60) | P-value |
|-------------------------|-----------------------|--------------------------|-----------------------------|---------|
| Age (years)             | 61 (51–71)            | 64 (49–70)               | 61 (51–72)                  | 0.88    |
| Female sex              | 27 (34)               | 8 (40)                   | 19 (32)                     | 0.49    |
| BMI (kg/m²)             | 28.4 (24.1–31.8)      | 23.3 (21.4–27.6)         | 30.1 (25.0–33.5)            | <0.001  |
| HF history >6 months    | 21 (26)               | 9 (45)                   | 12 (20)                     | 0.027   |
| Prior HF hospitalisation* | 30 (38)              | 9 (45)                   | 21 (35)                     | 0.42    |
| NYHA class II/III       | 51 (64)/29 (36)       | 15 (75)/5 (25)           | 36 (60)/24 (40)             | 0.23    |
| Systolic BP (mmHg)      | 105 (95–125)          | 101 (87–117)             | 105 (97–133)                | 0.13    |
| Hypertension            | 47 (59)               | 9 (45)                   | 38 (63)                     | 0.15    |
| Diabetes mellitus       | 22 (28)               | 3 (15)                   | 19 (32)                     | 0.15    |
| Treatment               |                       |                          |                             |         |
| ACEI/ARB/ARNI           | 65 (81)               | 15 (75)                  | 50 (83)                     | 0.41    |
| Beta-blocker            | 58 (73)               | 13 (65)                  | 45 (75)                     | 0.39    |
| MRA                     | 35 (44)               | 11 (55)                  | 24 (40)                     | 0.24    |
| Diuretic                | 52 (65)               | 16 (80)                  | 36 (60)                     | 0.10    |
| Oral anticoagulation    | 23 (29)               | 7 (35)                   | 16 (27)                     | 0.48    |
| Aspirin                 | 22 (28)               | 4 (20)                   | 18 (30)                     | 0.39    |
| Calcium antagonist       | 8 (10)                | 1 (5)                    | 7 (12)                      | 0.39    |
| Device therapy          | 9 (11)                | 3 (15)                   | 6 (10)                      | 0.54    |
| Laboratory parameters   |                       |                          |                             |         |
| Ferritin (µg/L)         | 154 (79–293)          | 170 (35–283)             | 141 (840–328)               | 0.34    |
| Iron (mg/L)             | 13.0 (10.0–17.8)      | 12.0 (8.5–15.8)          | 13.0 (10.0–18.0)            | 0.45    |
| TSAT (%)                | 23.0 (17.0–32.0)      | 23.0 (16.3–30.8)         | 23.0 (17.0–32.8)            | 0.71    |
| sTFR (µg/dL)            | 3.0 (2.4–4.2)         | 2.8 (2.5–6.1)            | 3.1 (2.4–4.2)               | 0.79    |
| Haemoglobin (g/dL)      | 13.4 (12.6–14.7)      | 13.0 (11.6–14.3)         | 13.6 (12.7–14.7)            | 0.15    |
| Anaemia                 | 22 (28)               | 7 (35)                   | 15 (25)                     | 0.39    |
| Hepcidin (ng/mL)        | 26.9 (8.3–45.3)       | 17.3 (2.3–40.5)          | 28.7 (9.2–45.9)             | 0.09    |
| eGFR (mL/min)           | 70.5 (56.3–88.0)      | 69.0 (50.3–87.5)         | 70.5 (57.3–88.0)            | 0.62    |
| CRP (mg/L)              | 3.9 (2.0–11.0)        | 3.4 (1.8–11.2)           | 4.0 (2.1–11.0)              | 0.64    |
| IL-6 (ng/L)             | 10.0 (4.3–18.8)       | 9.5 (3.5–18.0)           | 10.0 (4.3–19.0)             | 0.54    |
| hs-cTnT (ng/L)          | 27 (16–43)            | 32 (16–43)               | 26 (16–42)                  | 0.73    |
| GDF-15 (ng/L)           | 1786 (1035–2859)      | 2303 (1426–3228)         | 1635 (951–2750)             | 0.32    |
| NT-proBNP (ng/L)        | 1859 (698–3740)       | 3566 (1513–6412)         | 1542 (526–2811)             | 0.005   |

Data are n (%), or median (interquartile range).

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARNI, angiotensin receptor–neprilysin inhibitor; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor 15; HF, heart failure; hs-cTnT, high-sensitivity cardiac troponin T; IL-6, interleukin 6; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; Q₁, lowest quartile; Q₂–₄, upper three quartiles; sTFR, soluble transferin receptor; TSAT, transferrin saturation.

*No patient was previously hospitalised more than once.

CI (online supplementary Table S3). CI was not significantly different between patients who had or had not previously been hospitalised with decompensated HF and was not related to the time interval from decompensation (online supplementary Table S4).

Cl₂₁ patients had significantly higher NT-proBNP levels than Cl₂₋₄ patients [3566 (1513–6412) vs. 1542 (526–2811) ng/L; P = 0.005] (Figure 7). In a multivariate analysis that considered age, sex, BMI, HF symptom duration, and estimated GFR, Cl₂₁ patients had a 6.0-fold (95% confidence interval 1.5–24.1) higher risk of having an NT-proBNP concentration above the median than Cl₂₋₄ patients (P = 0.011).

When CI quartiles were analysed individually, Cl₂₁ patients had a significantly lower BMI than Cl₂₋₄ patients and significantly higher NT-proBNP levels than Cl₂₂ and Cl₂₃ patients (online supplementary Table S5).

Cardiac iron and endomyocardial biopsy analysis

Clinically, all patients presented with non-ischaemic HFrEF. EMB evaluation revealed non-ischaemic cardiomyopathy with no or low-grade inflammation in 34 patients, non-ischaemic cardiomyopathy with high-grade inflammation in 42 patients, active myocarditis in 2 patients, and amyloidosis in another 2 patients. CI was not related to EMB-based diagnostic subgroups (online supplementary Table S6). Average cardiomyocyte diameters, collagen type I and type III area fractions, and accumulation of...
CD3<sup>+</sup>, CD45R0<sup>+</sup>, LFA-1<sup>+</sup>, or MAC-1<sup>+</sup> inflammatory cells were not significantly different in CIQ1 or CIQ2–4 patients (Table 2). By linear regression analysis, CI was not associated with CD3<sup>+</sup>, CD45R0<sup>+</sup>, LFA-1<sup>+</sup>, or MAC-1<sup>+</sup> inflammatory cell numbers in the myocardium, whereas CI was weakly associated with collagen type III area fraction (online supplementary Figure S4). Genomes of adenovirus, Epstein–Barr virus, enterovirus, human herpesvirus 6, and parvovirus B19 were detected at similar frequencies in CIQ<sub>1</sub> and CIQ<sub>2–4</sub> patients (Table 2).

**Iron status and left ventricular remodelling**

Left ventricular end-diastolic and end-systolic diameter indices, LV volume indices, LVEF, LV end-diastolic pressure, LV dP/dt<sub>max</sub>, and interventricular septum thickness and LV mass indices were not significantly different in patients with or without systemic ID (online supplementary Table S7). Some of these echocardiographic parameters, however, aligned with CI. Specifically, CIQ<sub>1</sub> patients had greater LV end-diastolic [31 (29–36) vs. 27 (25–31) mm/m<sup>2</sup>; P = 0.001] and end-systolic diameter indices [27 (24–31) vs. 23 (20–27) mm/m<sup>2</sup>; P = 0.003] than CIQ<sub>2–4</sub> patients (Figure 2). LV end-diastolic and end-systolic volume indices were not significantly greater in CIQ<sub>1</sub> than in CIQ<sub>2–4</sub> patients; likewise, LVEF was not significantly different between the two groups [23 (17–28) vs. 27 (18–34); P = 0.15] (online supplementary Table S7). LV end-diastolic pressure was higher in CIQ<sub>1</sub> than in CIQ<sub>2–4</sub> patients [16 (11–21) vs. 11 (6–19) mmHg; P = 0.046] (Figure 2). When CI quartiles were analysed individually, CIQ<sub>1</sub> patients had significantly greater LV end-diastolic and end-systolic diameter indices than CIQ<sub>2–4</sub> patients (online supplementary Table S8).

### Discussion

This study is the first to measure CI in patients with non-advanced HF and to use LV EMIs as source material. We present two main findings: first, patients with low CI have greater disease severity and, second, CI is not related to systemic iron status.

Although we studied a relatively homogeneous population of patients presenting with non-ischaeimic HFrEF, CI varied widely in individual patients (range 132–1339 µg/g dry tissue). Lacking established normal values, we arbitrarily chose the lower quartile boundary to define patients with low CI. Patients with lower BMIs (still within the normal weight range in our population) and those with longer HF histories were more likely to have low CI. CI in patients with non-advanced HFrEF was approximately twofold higher than CI previously measured in patients with end-stage HF<sup>21,22</sup>. This difference does not appear to be related to the analytical methods (ICP-OES or ICP-MS) employed in these studies. While we cannot fully explain this difference, varied HF duration may have played

### Table 2 Immunohistology and viral polymerase chain reaction

|                     | All patients (n = 80) | Cardiac iron Q<sub>1</sub> (n = 20) | Cardiac iron Q<sub>2–4</sub> (n = 60) | P-value |
|---------------------|----------------------|-------------------------------------|-------------------------------------|---------|
| Cardiomyocyte diameter (µm) | 23 (20–25)          | 23 (20–25)                         | 23 (20–25)                         | 0.87    |
| Collagen type I area fraction (%) | 9.8 (2.7–21.3)      | 10.3 (2.4–21.4)                    | 10.9 (6.3–16.6)                    | 0.34    |
| Collagen type III area fraction (%) | 9.8 (6.2–16.9)     | 8.4 (4.4–18.1)                      | 10.7 (2.6–22.5)                    | 0.18    |
| CD3<sup>+</sup> cells (mm<sup>-2</sup>) | 9.8 (2.0–22.5)     | 4.3 (0.8–21.2)                      | 10.7 (2.6–22.5)                    | 0.18    |
| CD45R0<sup>+</sup> cells (mm<sup>-2</sup>) | 52.5 (33.0–72.5)   | 38.0 (27.0–67.0)                    | 53.3 (34.7–75.8)                    | 0.10    |
| LFA-1<sup>+</sup> cells (mm<sup>-2</sup>) | 16.7 (6.7–33.0)    | 11.1 (5.3–28.2)                     | 18.2 (8.2–37.1)                     | 0.16    |
| MAC-1<sup>+</sup> cells (mm<sup>-2</sup>) | 36.3 (20.4–61.7)   | 28.3 (17.6–49.6)                    | 40.9 (21.4–68.9)                    | 0.16    |
| Adenovirus detectable | 0                   | 0                                   | 1                                  | 1.0     |
| Epstein–Barr virus detectable | 0                   | 0                                   | 1                                  | 1.0     |
| Enterovirus detectable | 0                   | 0                                   | 1                                  | 1.0     |
| Human herpesvirus 6 detectable | 6 (8)              | 2 (10)                              | 4 (7)                              | 0.64    |
| Parvovirus B19 detectable | 51 (64)             | 14 (70)                             | 37 (62)                            | 0.60    |

Data are n (%), or median (interquartile range). Collagen type I area fraction (n = 56), collagen type III area fraction (n = 55). CD, cluster of differentiation; LFA-1, lymphocyte function-associated antigen 1; MAC-1, macrophage-1 antigen; Q<sub>1</sub>, lowest quartile; Q<sub>2–4</sub>, upper three quartiles.
Cardiac iron and left ventricular remodelling. (A) Left ventricular end-diastolic diameter (LVEDD) index, (B) left ventricular end-systolic diameter (LVESD) index, and (C) left ventricular end-diastolic pressure (LVEDP) in patients with cardiac iron concentrations in the lowest quartile (Q₁) or upper three quartiles (Q₂–Q₄). Individual data points, median, and the upper and lower quartile boundaries are shown.

Figure 2

a role. Indeed, we found HF duration to be independently associated with low CI, suggesting that cardiac ID may develop or worsen during the course of the disease.

We studied patients with non-ischaemic HFrEF some of who presented with myocardial inflammation as typically observed in patients clinically diagnosed with dilated cardiomyopathy undergoing EMB evaluation. Low CI was not related to EMB-based diagnostic subgroups, immunohistological findings (fibrosis, cardiomyocyte diameters, inflammation), or the presence of cardiotoxic viral genomes in the biopsies. It is possible that in pathophysiologic conditions associated with more pronounced myocardial inflammation (e.g. active myocarditis; not studied here) or in more heterogeneous patient populations presenting with low, intermediate, or very high levels of cardiac inflammation, CI will be associated with cardiac inflammation. Notably, low CI was also not associated with systemic ID, anaemia, or systemic iron homeostasis biomarkers, indicating that CI cannot be inferred from these routine blood measurements. These data add to a growing body of evidence that systemic iron homeostasis and CI are differentially regulated. We have previously shown in mice with post-infarction HF that iron-regulatory proteins 1 and 2 cell-autonomously secure iron availability in cardiomyocytes independent of systemic iron status. Rats with volume overload-induced HF have been shown to develop cardiac ID although iron absorption and systemic iron levels are preserved. In a study of 33 patients with advanced HF, serum iron markers did not reflect LV myocardial iron levels, except for sTFR which tended to display a negative correlation.

Based on higher serum NT-proBNP levels, LV end-diastolic and end-systolic diameters, and LV end-diastolic pressure, patients with low CI had more severe disease than patients with higher CI. Increased LV diameters did not translate into significantly greater LV volumes (although, numerically, LV volumes were larger and LVEF was smaller in patients with low CI). Compared with LV diameters, which are a highly reproducible measure of LV size, measurements of LV volumes may be less accurate in some patients, especially when endocardial borders are not well defined and/or if imaging quality is affected by body habitus. A previous study in 33 patients found no association between CI and NT-proBNP. Another study in 91 patients reported no association between CI and BNP, LV end-diastolic diameter, or LVEF. Both studies, however, investigated heterogeneous patient populations with advanced HF, mostly related to ischaemic cardiomyopathy, and with much higher (NT-pro)BNP levels than in our population.

Our study cannot establish a causal relationship between low CI and increased disease severity. In other words, it remains to be investigated if CI is a marker of disease severity and/or a factor contributing to disease progression in patients with HF. Experimental studies indicate that low CI can directly promote contractile dysfunction and HF. Indeed, mice with genetically-engineered cardiomyocyte-specific ID develop HF due to impaired mitochondrial respiration and disturbed cardiac energy reserve. Notably, depleting intracellular iron also impairs mitochondrial function and contractility in human-induced pluripotent stem cell-derived cardiomyocytes.

In the future, patients with low CI and a preserved systemic iron status may be candidates for iron supplementation therapy. In mice with genetically-engineered cardiomyocyte-specific ID, intravenous iron supplementation increases CI even when cellular iron regulation is defective, e.g. by deletion of iron regulatory proteins or the transferrin receptor in cardiomyocytes. This, however, remains to be shown in patients. It will also be interesting to explore if improvements in symptoms and exercise tolerance by iron supplementation relate to cardiac and/or peripheral iron repletion.

Our study has limitations that merit consideration. First, CI in LV EMBs reflects iron levels not only in cardiomyocytes but also in other cellular constituents. In mice genetically engineered to develop cardiomyocyte-selective ID, a ∼30% reduction in cardiomyocyte iron resulted in a ∼30% reduction of total LV myocardial iron, suggesting that cardiac iron is mainly localised within cardiomyocytes. CI was not related to LV myocardial inflammation, interstitial fibrosis, or cardiomyocyte hypertrophy in our study, thereby showing that interindividual variations in
Cl were not related to differences in tissue composition in our patients (this may be different in other HF aetiologies, e.g. in ischaemic cardiomyopathy with more pronounced and variable intramyocardial scarring). We therefore believe that in our study, Cl primarily reflects iron localised within cardiomyocytes. Second, we are unable to define a reference range for CI. For ethical reasons, we could not obtain EMBs from healthy individuals; non-transplanted donor hearts were not investigated as they differ from EMBs in tissue composition and pre-analytic handling. Third, we investigated younger patients with non-ischaemic HFrEF and a low number of comorbidities. Therefore, our findings need to be validated in other HF aetiologies and patient cohorts. Fourth, our cohort includes a few patients with active myocarditis (n = 2) and amyloidosis (n = 2). After excluding these patients, low CI remained significantly associated with higher NT-proBNP levels and larger LV diameter indices (online supplementary Table S9).

In conclusion, patients with non-advanced non-ischaemic HFrEF and low CI have more severe disease. CI needs to be directly measured and cannot be predicted based on systemic iron status (Figure 3). Future studies should explore whether low CI influences outcome in HFrEF and analyse the effects of iron supplementation therapy in patients with low CI.

**Supplementary Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Acknowledgement**

We thank Ivonne Marquardt for assistance in patient screening and recruitment.

**Funding**

This work was supported by the German Research Foundation [Clinical Research Unit (KFO311) to T.K., J.B., and K.C.W.] and an unrestricted research grant from Vifor Pharma Ltd. to TK.

**Conflict of interest:** T.K. has received an unrestricted research grant and advisory board and speaker fees from Vifor Pharma Ltd. J.B. has received consulting and speaker fees from Vifor Pharma Ltd. All other authors report no relationships relevant to this study.

© 2020 The Authors. European Journal of Heart Failure published by John Wiley & Sons Ltd on behalf of European Society of Cardiology.
Cardiac iron in non-ischaemic heart failure

References

1. Anand IS, Gupta P. Anemia and iron deficiency in heart failure: current concepts and emerging therapies. Circulation 2018;138:80–98.
2. McDonagh T, Damy T, Doehner W, Lam CS, Sindone A, van der Meer P, Cohen-Solal A, Kindermann I, Manito N, Pfister O, Pohjantahalli-Maaras H, Taylor J. Comin-Colet J. Screening, diagnosis and treatment of iron deficiency in chronic heart failure: putting the 2016 European Society of Cardiology heart failure guidelines into clinical practice. Eur J Heart Fail 2018;20:1664–1672.
3. Rocha BM, Cunha DJ, Menezes Falcao LF. The burden of iron deficiency in heart failure: therapeutic approach. J Am Coll Cardiol 2018;71:782–793.
4. Jankowska EA, Roche BM, Cunha GJ, Menezes Falcao LF. The burden of iron deficiency in heart failure: current concepts and management. J Cardiovasc Med 2018;19:899–906.
5. Kott JC, Comin-Colet J, Voors AA, Ponikowski P, Enjueses C, Banasiak W, Van der Meer P, Jankowska E. Iron deficiency in chronic heart failure: an international pooled analysis. Am Heart J 2013;165:575–582.e3.
6. Enjueses C, Klip IT, Brugueria J, Cladellas M, Ponikowski P, Banasiak W, van Veldhuisen DJ, van der Meer P, Jankowska E. 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.
7. von Haelming S, Gremmiller U, Krumm M, Mibach F, Schön N, Taggeselle J, Dahm JB, Angermann CE. Prevalence and clinical impact of iron deficiency and anemia among outpatients with chronic heart failure: the PRÉP registry. Clin Res Cardiol 2017;106:436–443.
8. Jankowska EA, Rozentryt P, Widowski A, Nowak J, Hartmann O, Ponikowski P, Borodulin-Nadzieja L, Banasiak W, Banasiak S, Filippatos G, McMurray JJ, Anker SD. Iron deficiency: an ominous sign in patients with chronic systolic heart failure. Eur Heart J 2010;31:1872–1880.
9. Jankowska EA, Małyszko J, Ardehali H, Koc-Zorawska E, Banasiak W, van Haelshing S, Macdougall IC, Weiss G, McMurray JJ, Anker SD. Geroheartiang M, Ponikowski P. Iron status in patients with chronic heart failure. Eur Heart J 2013;34:827–834.
10. Jankowska EA, Kasztura M, Sokolski M, Bronisz M, Nawrocka S, Oleksyka-Florcz M, Zymilinski R, Biegus J, Siwolowski P, Banasiak W, Anker SD, Filippatos G, Cleland JG, Ponikowski P. Iron deficiency defined as depleted iron stores accompanied by unmet cellular iron requirements identifies patients at the highest risk of death after an episode of acute heart failure. Eur Heart J 2014;35:2468–2476.
11. Martinez P, Verbrugge FH, Nijst P, Dupont M, Mullens W. Limited contractile reserve contributes to poor peak exercise capacity in iron-deficient heart failure. Eur J Heart Fail 2018;20:806–808.
12. Anker SD, Comin-Colet J, Filippatos G, Willenheimer R, Dickstein K, Drezer H, Lüscher TF, Bart B, Banasiak W, Niegosj J, Werni BA, Mori C, von 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;361:2431–2440.
13. Ponikowski P, van Veldhuisen DJ, Comin-Colet J, Erd G, Komajda M, Mareev V, McDonagh T, Parkhomkeno A, Tavazzi L, Levesque V, Mori C, Roubert B, Filippatos G, Ruczyńska F, Anker SD. CONFIRM-HF Investigators. Beneficial effects of long-term intravenous iron therapy with ferric carboxymaltose in patients with symptomatic heart failure and iron deficiency. Eur Heart J 2013;36:657–668.
14. van Veldhuisen DJ, Ponikowski P, van der Meer P, Metra M, Böhm M, Dolecky S, Voors AA, Macdougall IC, Anker SD, Roubert B, Zaklin L, Cohen-Solal A. EFFECT-HF Investigators. Effect of ferric carboxymaltose on exercise capacity in patients with chronic heart failure and iron deficiency. Circulation 2017;136:1374–1383.
15. Ponikowski P, Voors AA, Pfeffer MA, Bakris GL, Anker SD, Hochmann S, Coads AJ, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nilsson-annopoulou P, Parissis JT, Pieske B, Riley JP, Rosano GM, Rutlege LM, Ruchitsch F, Ruten FH, van der Meer P. 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). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016;18:991–975.
32. Kühl U, Pauschinger M, Seeberg B, Lassner D, Noutsias M, Poller W, Schultheiss HP. Viral persistence in the myocardium is associated with progressive cardiac dysfunction. Circulation 2005;112:1965–1970.
33. Katzmann JI, Schlattmann P, Rigopoulos AG, Noutsias E, Bigalke B, Pauschinger M, Tschoppe C, Sedding D, Schulze PC, Noutsias M. Meta-analysis on the immunohistological detection of inflammatory cardiomyopathy in endomyocardial biopsies. Heart Fail Rev 2020;25:277–294. https://doi.org/10.1007/s10741-019-09835-9.
34. Nakayama T, Sugano Y, Yokokawa T, Nagai T, Matsuyama TA, Ohta-Ogo K, Ikeda Y, Ishibashi-Ueda H, Nakatani T, Ohto N, Yasuda S, Anzai T. Clinical impact of the presence of macrophages in endomyocardial biopsies of patients with dilated cardiomyopathy. Eur J Heart Fail 2017;19:490–498.
35. Petrak J, Havlenova T, Krijt M, Behounek M, Frankova J, Cervenka L, Pluhacek T, Vyoral D, Melenovsky V. Myocardial iron homeostasis and hepcidin expression in a rat model of heart failure at different levels of dietary iron intake. Biochim Biophys Acta Gen Subj 2019;1863:703–713.
36. Leszek P, Sochanowicz B, Brzoska K, Danko B, Kraj L, Kusmierczyk M, Piotrowski W, Sobieszczanska-Malek M, Rywik TM, Polkowska-Motrenko H, Kruszewski M. Does myocardial iron load determine the severity of heart insufficiency? Int J Cardiol 2015;182:191–193.
37. Hoes MF, Grote Beverborg N, Kijlstra 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.
38. Melenovsky V, Hlavata K, Sedivy P, Dezortova M, Borlaug BA, Petrak J, Kautzner J, Hajek M. Skeletal muscle abnormalities and iron deficiency in chronic heart failure. An exercise 31P magnetic resonance spectroscopy study of calf muscle. Circ Heart Fail 2018;11:e004800.
39. Charles-Edwards G, Amaral N, Sleigh A, Ays S, Catibog N, McDonagh T, Monaghan M, Amin-Youssef G, Kemp GJ, Shah AM, Okonko DO. Effect of iron isomaltoside on skeletal muscle energetics in patients with chronic heart failure and iron deficiency. Circulation 2019;139:2386–2398.