


Impact of change in iron status over time on clinical outcomes in heart failure according to ejection fraction phenotype

Sarah Fitzsimons^{1*} , Tee Joo Yeo^{2,3}, Lieng H. Ling^{2,3}, David Sim³, Kui Toh Gerard Leong⁴, Poh Shuan Daniel Yeo⁵, Hean Yee Ong⁶, Fazlur Jaufeerally⁷, Tze P. Ng⁸, Katrina Poppe¹, Mayanna Lund⁹, Gerry Devlin¹⁰, Richard Troughton¹¹, Carolyn S.P. Lam^{2,3}, A. Mark Richards^{2,8,11} and Robert N. Doughty¹

¹Heart Health Research Group, University of Auckland, Auckland, New Zealand; ²Cardiovascular Research Institute, National University Health System, Singapore; ³National University Heart Centre Singapore, Singapore; ⁴Changi General Hospital, Singapore; ⁵Tan Tock Seng Hospital, Singapore; ⁶Khoo Teck Puat Hospital, Singapore; ⁷Singapore General Hospital, Singapore; ⁸Yong Loo Lin School of Medicine, National University of Singapore, Singapore; ⁹Middlemore Hospital, Auckland, New Zealand; ¹⁰Waikato Hospital, Hamilton, New Zealand; and ¹¹Christchurch Heart Institute, University of Otago, Christchurch, New Zealand

Abstract

Aims The importance of iron deficiency (ID) in heart failure with preserved ejection fraction (HFpEF) is unknown. In HF with reduced ejection fraction (HFrEF), ID is reported as an independent predictor of mortality in HF although not all published studies agree. Different definitions of ID have been assessed, and the natural history of untreated ID not established, which may explain the conflicting results. This study aimed to assess the relationship between ID and mortality in HFpEF, clarify which definition of ID correlates best with outcomes in HFrEF, and determine the prognostic importance of change in ID status over time.

Methods and results Analyses were conducted on data from 1563 patients participating in a prospective international cohort study comparing HFpEF with HFrEF. Plasma samples from baseline and 6 month visits were analysed for the presence of ID. Two ID definitions were evaluated: ID_{Ferritin} = 'ferritin < 100 mcg/L or ferritin 100–300 mcg/L + transferrin saturation < 20%' and ID_{Tsat} = 'transferrin saturation < 20%'. The risk of all-cause mortality and death/HF hospitalization associated with baseline ID (ID_{Ferritin} or ID_{Tsat}) and change in ID status at 6 months (persistent, resolving, developing, or never present) was estimated in multivariable Cox proportional hazards models. Of 1563 patients, 1115 (71%) had HFrEF and 448 (29%) HFpEF. Prevalence of ID was similar in HFpEF and HFrEF (58%). Patients with ID were more likely to be female, diabetic, and have a higher co-morbid burden than patients without ID. ID by either definition did not confer independent risk for either all-cause mortality or death/HF hospitalization for patients with HFpEF [ID_{Ferritin} hazard ratio (HR) 0.65 (95% confidence interval 0.40–1.05), *P* = 0.08; ID_{Tsat} HR 1.16 (0.72–1.87), *P* = 0.55]. In the overall study cohort (HFrEF + HFpEF) and HFrEF subgroup, ID_{Ferritin} was inferior to ID_{Tsat} in prediction of all-cause mortality [overall cohort: HR 1.21 (0.95–1.53), *P* = 0.12 vs. HR 1.95 (1.52–2.51), *P* < 0.01; HFrEF: HR 1.12 (0.85–1.48), *P* = 0.43 vs. HR 1.57 (1.15–2.14), *P* < 0.01]. Persistence of ID_{Tsat} at 6 months was strongly associated with poor outcomes compared with never having ID_{Tsat} [HR 2.22 (1.42–3.46), *P* < 0.01] or having ID_{Tsat} at baseline self-resolve by 6 months [HR 1.40 (1.06–1.86), *P* = 0.02].

Conclusions Iron deficiency is equally prevalent in HFpEF and HFrEF but is negatively prognostic only in HFrEF. The natural history of ID is important; persistent ID is strongly associated with mortality whereas resolution is not. ID_{Tsat} is the superior definition of ID and should inform future trials investigating the efficacy of intravenous iron replacement in patients with HFrEF.

Keywords Heart failure; Heart failure with preserved ejection fraction; Iron deficiency; Mortality; Rehospitalization

Received: 8 April 2021; Revised: 19 August 2021; Accepted: 4 September 2021

*Correspondence to: Sarah Fitzsimons, Heart Health Research Group, School of Medicine, University of Auckland, Park St Grafton, Auckland, 1023 New Zealand.
Tel: +6499239804; Fax: +6493677146. Email: sfitzsimons@adhb.govt.nz

Introduction

Iron deficiency (ID) is recognized as an important co-morbidity in patients with heart failure (HF). A high prevalence of ID has been found in patients with HF with reduced ejection fraction (HFrEF).^{1–3} Whether this is true for patients with HF with preserved ejection fraction (HFpEF) is not well established, as studies published to date have either excluded HFpEF patients or included insufficient numbers for definitive conclusions. ID is reported as an independent predictor of mortality in HF although not all published studies agree.^{1–6} Different definitions of ID have been used, which may explain the conflicting results. ID defined as ‘ferritin < 100 or 100–300 mcg/L with a transferrin saturation (tsat) < 20%’ are the criteria most widely studied and were used in the randomized controlled trials underpinning guideline recommendations for intravenous (IV) iron replacement in HF patients with ID. However, when correlated with the ‘gold standard’ definition of ID [bone marrow (BM) stores], ferritin < 100 or 100–300 mcg/L with a tsat < 20% was found to be inferior to other definitions of ID.⁷ Furthermore, the natural history of untreated ID in HF and its prognostic implications have not been established.

Aims

- 1 Quantify prevalence of ID and determine whether ID predicts long-term outcomes in HFpEF as compared with HFrEF.
- 2 Clarify which definition of ID is more closely related to all-cause mortality and the secondary combined endpoint of death/HF hospitalization.
- 3 Determine whether change in ID status over time is an important prognostic factor.

Methods

Patient cohort

Patients were a subset of those enrolled in a prospective, longitudinal multicentre study carried out in New Zealand (four centres) and Singapore (six centres) to determine clinical outcomes and predictors of outcome in HFpEF compared with HFrEF. The study design and main outcomes have been previously published.⁸ In brief, patients with clinically confirmed HF (both inpatients and outpatients) were prospectively identified between March 2010 and August 2014 for inclusion in this study, a clinical era pre-dating the widespread use of IV iron replacement in HF. Patients were assessed at recruitment (baseline visit), 6 weeks, and 6 months after enrolment, and clinical outcomes were determined up to 2 years following enrolment. The diagnosis of HF satisfied European Society

of Cardiology criteria at the time of data collection (2012 Guidelines). Left ventricular ejection fraction (LVEF) <50% and ≥50%, determined quantitatively on transthoracic echocardiogram, defined HFrEF and HFpEF, respectively. All patients who had serum samples stored at their baseline visit were included. Informed consent had been obtained from all patients for future studies on their stored blood samples. Our study complies with the Declaration of Helsinki, and our research protocol was approved by all local ethics committees.

Evaluation of iron deficiency

Blood samples were drawn at baseline and 6 month visits, plasma separated by 10 min centrifugation at 4000 rpm at 4°C, aliquoted into EDTA, and then frozen at –80°C. Haemoglobin (Hb) was measured using a standard Seismic assay, and iron, ferritin, and transferrin levels were measured on the Abbott Architect platform. Tsat was calculated as the ratio of serum iron (mg/L) to total iron binding capacity (mg/L) × 100.

Our primary definition of ID (ID_{Ferritin}) was a ‘ferritin level < 100 mcg/L’ (absolute ID) or ‘ferritin 100–300 mcg/L with a tsat < 20%’ (functional ID). This was chosen as, to date, it is the most widely assessed definition of ID in HF patients.^{9,10} We also assessed a second definition of ID —‘tsat < 20%’ (ID_{Tsat}). This was chosen as it was the definition of ID most closely reflective of BM iron stores, the gold standard definition, that is also available in routine clinical care worldwide.⁷

Patients who had ID at both their baseline and 6 month visits were designated as having ‘persistent ID’. Patients with ID at baseline but not at 6 months were labelled ‘resolved ID’. Patients without ID at baseline but with ID at 6 months had ‘developed ID’, and, finally, those free of ID at either time point were classified ‘never ID’.

Statistical analysis

Statistical analyses were performed using R software (R Core Team 2017). Statistical tests are two-sided, and $P < 0.05$ was considered significant. Normally distributed data are presented as mean (standard deviation), whereas categorical variables are expressed as numbers (percentages). Comparisons of categorical variables were performed using a χ^2 or Fisher’s exact test, as required. Multivariable Cox proportional hazards models were used to estimate the adjusted risk associated with baseline ID (ID_{Ferritin} or ID_{Tsat}) and outcomes including all-cause mortality and death/rehospitalization. Covariates were selected a priori as the clinical and laboratory variables established as independent predictors of all-cause mortality and death or HF readmission in the parent study.⁸ These were

age, gender, ischaemic aetiology, N-terminal pro-brain natriuretic peptide (NT-proBNP), New York Heart Association (NYHA) class, systolic blood pressure (SBP), atrial fibrillation (AF), and serum creatinine.⁸ To assess the effect of change in ID status at 6 months (persistent, resolving, developing, or never present), we developed proportional hazard models that adjusted only for change in ID status (Model 1); for change in ID status and EF group (Model 2); for change in ID status, EF group, age, and gender (Model 3); and for change in ID status, age, gender, $\Delta\log\text{Creat}$, and $\Delta\log\text{NT-proBNP}$ (Model 4).

Results

Baseline characteristics

Baseline characteristics of participants according to LVEF category are given in *Table 1*. There were 1565/2039 (77%) patients with adequate stored plasma samples of whom 1563 had LVEF measured; 1115 (71%) had HFrEF and 448 (29%) HFpEF. ID_{Ferritin} classified 900 (58%) patients as ID; of these, 546 patients had absolute ID and 354 functional ID. Prevalence of ID_{Ferritin} was similar in HFpEF and HFrEF patients (58%). Absolute ID was the cause of ID in 64% of HFpEF and 59% of HFrEF patients. When ID was defined as ID_{Tsat}, 902 (58%) patients were classified as ID. Prevalence of ID_{Tsat} was also similar in HFpEF and HFrEF patients (57% vs. 58%).

Characteristics of iron deficiency compared with non-iron deficiency patients

Within our study population, patients with ID_{Ferritin} were more likely to be female (33% vs. 21%, $P < 0.0001$), diabetic (48% vs. 38%, $P = 0.0003$), and have a higher co-morbid burden than patients without ID_{Ferritin}. There was a small difference in clinical markers of HF including more patients with NYHA class III/IV symptoms ($P = 0.046$) and a higher average plasma NT-proBNP in those with ID_{Ferritin}. The use of anti-coagulant (30% ID_{Ferritin}, 28% non-ID_{Ferritin}, $P = 0.65$), anti-platelet (42% ID_{Ferritin}, 38% non-ID_{Ferritin}, $P = 0.20$), and oral iron replacement medications (24% ID_{Ferritin}, 27% non-ID_{Ferritin}, $P = 0.12$) was high and did not differ significantly by iron status. More patients with ID_{Ferritin} had a history of peptic ulceration (7% vs. 4%, $P = 0.034$).

There was no significant difference in baseline characteristics between patients defined as ID_{Tsat} vs. ID_{Ferritin} (Supporting Information, *Table S1*). Differences between ID_{Tsat} and non-ID_{Tsat} were similar to between ID_{Ferritin} and non-ID_{Ferritin} except a history of peptic ulceration was not more common in the ID group (6% vs. 5%, $P = 0.43$).

Characteristics of patients with heart failure with preserved ejection fraction (iron deficiency vs. non-iron deficiency)

The difference in co-morbid burden and clinical markers of HF between those with and without ID was more pronounced in HFpEF patients (*Table 1*). Patients with HFpEF and ID_{Ferritin} had higher prevalence of AF (60% vs. 45%, $P = 0.003$), hypertension (80% vs. 71%, $P = 0.030$), and NYHA III–IV HF (31% vs. 19%, $P = 0.004$) than HFpEF patients without ID_{Ferritin}. Proportionally, more HFpEF patients with ID_{Ferritin} were prescribed anti-coagulant or anti-platelet agents than HFpEF patients without ID_{Ferritin} and HFrEF patients with or without ID_{Ferritin}. Similar differences were present between ID and non-ID HFpEF patients when ID was defined as ID_{Tsat}.

Iron deficiency as a predictor of all-cause mortality

During 2 years of follow-up, 293/1563 (19%) patients died from any cause. There were 179 deaths in those with ID_{Ferritin} compared with 114 deaths in those without. In a model adjusting for age and sex, there was no significant difference in all-cause mortality between patients with ID_{Ferritin} at baseline vs. those without {hazard ratio [HR] 1.21 [confidence interval (CI) 0.95–1.53], $P = 0.12$ } (*Table 2, Figure 1*).

When ID was defined by ID_{Tsat}, there were 206 (23%) deaths in those with ID_{Tsat} compared with 179 (20%) in the group identified as ID_{Ferritin}. In contrast to ID_{Ferritin}, ID_{Tsat} was an independent risk factor for all-cause mortality in a model adjusted for age and sex [HR 1.95 (1.52–2.51), $P < 0.01$]. When age, sex, ischaemic aetiology, NYHA class, SBP, AF, log(NT-proBNP), and log(creatinine) were included, ID_{Tsat} remained an independent predictor for all-cause mortality [adjusted HR 1.46 (1.12–1.89), $P < 0.01$].

Iron deficiency as a predictor of death and heart failure hospitalization

During 2 years of follow-up, 677 (43%) patients either died or incurred worsening HF requiring HF hospitalization, including 417 (46%) patients with ID_{Ferritin} and 260 (39%) without. Cox proportional hazard modelling including age and sex demonstrated a significant difference in the risk of death or hospitalization between those with and without ID_{Ferritin}, which was not maintained when ischaemic aetiology, NYHA class, SBP, AF, log(NT-proBNP), and log(creatinine) were included in the model [HR 1.07 (0.92–1.26), $P = 0.52$] (*Table 2, Figure 2*). In contrast, ID_{Tsat} remained a significant risk factor for death/HF hospitalization [HR 1.19 (1.01–1.41), $P = 0.03$] when the model was adjusted for age, sex, ischaemic aetiology, NYHA class, SBP, AF, log(NT-proBNP), and log(creatinine).

Table 1 Baseline characteristics by ejection fraction and ID_{Ferritin} status

	HFpEF n = 448		HFREF N = 1115		Total cohort N = 1563	
	ID _{Ferritin} n = 258	Not ID _{Ferritin} n = 190	ID _{Ferritin} n = 642	Not ID _{Ferritin} n = 473	ID _{Ferritin} n = 900	Not ID _{Ferritin} n = 663
Age, years	74 (11)	70 (11)	63 (13)	63 (14)	66 (13)	65 (13)
Female, n (%)	143 (55)	72 (38)	154 (24)	66 (14)	297 (33)	138 (21)
BMI, kg/m ²	30 (8)	29 (6)	28 (7)	28 (7)	28 (7)	28 (7)
AF/flutter, n (%)	154 (60)	86 (45)	222 (35)	180 (38)	376 (42)	266 (40)
DM, n (%)	114 (44)	80 (42)	315 (49)	174 (37)	429 (48)	254 (38)
HTN, n (%)	206 (80)	134 (71)	427 (67)	282 (60)	633 (70)	416 (63)
PUD, n (%)	26 (10)	11 (6)	36 (6)	17 (4)	62 (7)	28 (4)
NYHA III/IV, n (%)	81 (31)	36 (19)	181 (28)	126 (27)	262 (29)	162 (24)
Hx HF hosp < 6/12, n (%)	50 (19)	42 (22)	221 (34)	136 (29)	271 (30)	178 (27)
HR, b.p.m.	70 (60, 80)	68 (61, 78)	76 (67, 86)	74 (65, 83)	75 (64, 84)	72 (64, 81)
SBP, mmHg	129 (115, 140)	129 (110, 142)	118 (104, 130)	118 (105, 132)	120 (107, 135)	120 (108, 136)
DBP, mmHg	70 (60, 78)	70 (61, 80)	70 (60, 79)	70 (62, 80)	70 (60, 79)	70 (62, 80)
NT-proBNP, pg/mL	1453 (710, 2693)	969 (375, 2376)	2513 (1342, 5057)	1756 (824, 3932)	2203 (1054, 4264)	1599 (606, 3597)
hsTnT, ng/L	26 (16, 44)	23 (13, 40)	33 (20, 52)	26 (16, 45)	30 (19, 50)	25 (15, 43)
LVEF (%)	61 (7)	60 (6)	29 (9)	30 (9)	38 (17)	38 (16)
E:E'	15 (8)	13 (7)	18 (8)	17 (8)	17 (8)	16 (8)
Creatinine, μmol/L	106 (84, 138)	109 (84, 143)	107 (88, 136)	104 (87, 128)	107 (87, 137)	105 (86, 135)
eGFR, mL/min/1.73 m ²	54 (40, 69)	57 (40, 76)	60 (45, 77)	64 (48, 80)	59 (44, 75)	62 (46, 79)
Hb, g/dL	121 (18)	129 (22)	131 (19)	139 (19)	128 (19)	136 (21)
HCT	0.38 (0.06)	0.4 (0.06)	0.4 (0.06)	0.42 (0.06)	0.4 (0.06)	0.41 (0.06)
Ferritin, mcg/L	78 (47, 126)	310 (188, 459)	85 (51, 139)	315 (189, 461)	81 (49, 136)	313 (189, 460)
Iron, mg/L	10 (5)	14 (6)	10 (5)	16 (8)	10 (5)	16 (7)
% saturation	15 (7)	25 (9)	15 (8)	27 (13)	15 (7)	27 (12)
Anaemia, n (%)	144 (56)	74 (39)	251 (39)	119 (25)	395 (44)	193 (29)
Anti-coag use, n (%)	92 (36)	57 (30)	174 (27)	131 (28)	266 (30)	188 (28)
Anti-plt use, n (%)	121 (47)	63 (33)	255 (40)	192 (41)	376 (42)	255 (38)
Iron supp, n (%)	54 (21)	39 (21)	158 (25)	141 (30)	212 (24)	180 (27)

AF, atrial fibrillation; BMI, body mass index; BP, blood pressure; coag, coagulant; DBP, diastolic blood pressure; DM, diabetes mellitus; E:E', mitral Doppler early velocity/mitral annular early velocity; eGFR, estimated glomerular filtration rate; Hb, haemoglobin; HCT, haematocrit; HR, heart rate; hsTnT, high-sensitivity troponin T; HTN, hypertension; Hx HF hosp, history of heart failure hospitalization; ID, iron deficiency; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association functional class; plt, platelet; PUD, peptic ulcer disease; sat, saturation; SBP, systolic blood pressure; supp, supplement.

Values are mean (standard deviation) or median (25% and 75% quartiles).

Table 2 Multivariable associations with outcome [hazard ratio (95% confidence interval)]

	HFpEF N = 448	HFrEF N = 1115	Total cohort N = 1563
<i>Hazard ratio for all-cause mortality (age/gender)</i>			
ID _{Ferritin}	0.65 (0.40–1.05)	1.41 (1.07–1.86)	1.21 (0.95–1.53)
Age	1.09 (1.06–1.12)	1.04 (1.03–1.05)	1.04 (1.03–1.05)
Gender (F:M)	0.83 (0.52–1.34)	0.59 (0.40–0.86)	0.59 (0.45–0.78)
ID _{Tsat}	1.16 (0.72–1.87)	2.31 (1.71–3.11)	1.95 (1.52–2.5)
Age	1.08 (1.05–1.11)	1.04 (1.03–1.06)	1.04 (1.03–1.05)
Gender (F:M)	0.77 (0.48–1.23)	0.58 (0.4–0.84)	0.57 (0.43–0.75)
<i>Hazard ratio for all-cause mortality (all independent variables)</i>			
ID _{Ferritin}	0.68 (0.41–1.12)	1.12 (0.85–1.48)	1.00 (0.78–1.27)
Age	1.06 (1.03–1.11)	1.02 (1.01–1.03)	1.03 (1.02–1.04)
Gender (F:M)	0.82 (0.49–1.40)	0.75 (0.51–1.11)	0.84 (0.62–1.12)
Ischaemic aetiology	1.13 (0.69–1.86)	1.82 (1.32–2.52)	1.47 (1.13–1.90)
Log_NT-proBNP	1.80 (1.39–2.32)	1.68 (1.45–1.93)	1.69 (1.50–1.89)
NYHA	1.47 (0.89–2.41)	1.71 (1.29–2.26)	1.69 (1.33–2.15)
SBP 10 mmHg	0.90 (0.80–1.01)	0.86 (0.80–0.93)	0.88 (0.82–0.93)
AF	0.75 (0.44–1.28)	1.30 (0.97–1.74)	1.11 (0.86–1.43)
logCreatinine	1.22 (0.62–2.38)	1.33 (0.88–2.0)	1.36 (0.96–1.92)
ID _{Tsat}	1.12 (0.69–1.84)	1.57 (1.15–2.14)	1.46 (1.12–1.89)
Age	1.06 (1.03–1.09)	1.02 (1.01–1.03)	1.03 (1.02–1.04)
Gender (F:M)	0.77 (0.46–1.29)	0.74 (0.50–1.10)	0.81 (0.6–1.08)
Ischaemic aetiology	1.05 (0.65–1.71)	1.78 (1.29–2.49)	1.45 (1.12–1.88)
Log_NT-proBNP	1.82 (1.40–2.36)	1.61 (1.40–1.86)	1.65 (1.46–1.84)
NYHA	1.47 (0.89–2.41)	1.64 (1.24–2.18)	1.65 (1.29–2.10)
SBP 10 mmHg	0.89 (0.80–1.00)	0.86 (0.80–0.93)	0.88 (0.83–0.93)
AF	0.70 (0.41–1.18)	1.31 (0.98–1.76)	1.12 (0.87–1.45)
logCreatinine	1.22 (0.62–2.40)	1.36 (0.90–2.06)	1.37 (0.97–1.94)
<i>Hazard ratio for death or HF hospitalization (age/gender)</i>			
ID _{Ferritin}	1.11 (0.81–1.52)	1.33 (1.11–1.60)	1.29 (1.11–1.51)
Age	1.03 (1.01–1.04)	1.02 (1.01–1.03)	1.01 (1.01–1.02)
Gender (F:M)	0.90 (0.66–1.23)	0.67 (0.53–0.85)	0.69 (0.58–0.83)
ID _{Tsat}	1.19 (0.87–1.63)	1.66 (1.38–1.99)	1.54 (1.31–1.80)
Age	1.03 (1.01–1.04)	1.02 (1.01–1.02)	1.01 (1.01–1.02)
Gender (F:M)	0.89 (0.65–1.22)	0.68 (0.53–0.86)	0.69 (0.57–0.82)
<i>Hazard ratio for death or HF hospitalization (all independent variables)</i>			
ID _{Ferritin}	0.99 (0.71–1.37)	1.11 (0.92–1.33)	1.07 (0.92–1.26)
Age	1.00 (0.99–1.02)	1.00 (0.99–1.01)	1.00 (0.99–1.01)
Gender (F:M)	0.97 (0.69–1.37)	0.82 (0.64–1.05)	0.89 (0.74–1.08)
Ischaemic aetiology	1.33 (0.96–1.84)	1.52 (1.24–1.86)	1.44 (1.22–1.71)
Log_NT-proBNP	1.56 (1.33–1.83)	1.37 (1.25–1.50)	1.41 (1.31–1.51)
NYHA	1.39 (1.00–1.95)	1.54 (1.27–1.86)	1.50 (1.27–1.77)
SBP 10 mmHg	0.94 (0.87–1.01)	0.96 (0.92–1.01)	0.96 (0.93–1.00)
AF	0.97 (0.69–1.38)	1.04 (0.85–1.27)	1.03 (0.87–1.22)
logCreatinine	1.37 (0.89–2.10)	1.41 (1.09–1.83)	1.42 (1.15–1.77)
ID _{Tsat}	1.06 (0.77–1.46)	1.24 (1.02–1.50)	1.19 (1.01–1.41)
Age	1.00 (0.99–1.02)	1.00 (0.99–1.01)	1.00 (1.00–1.01)
Gender (F:M)	0.96 (0.69–1.35)	0.82 (0.64–1.05)	0.89 (0.74–1.08)
Ischaemic aetiology	1.32 (0.96–1.82)	1.50 (1.23–1.84)	1.44 (1.21–1.70)
Log_NT-proBNP	1.56 (1.33–1.82)	1.35 (1.23–1.48)	1.39 (1.29–1.50)
NYHA	1.39 (1.00–1.95)	1.51 (1.25–1.84)	1.49 (1.26–1.75)
SBP 10 mmHg	0.94 (0.87–1.01)	0.96 (0.92–1.01)	0.96 (0.93–0.99)
AF	0.97 (0.69–1.37)	1.04 (0.85–1.26)	1.04 (0.87–1.23)
logCreatinine	1.37 (0.90–2.10)	1.43 (1.10–1.85)	1.43 (1.15–1.77)

AF, atrial fibrillation; Hb, haemoglobin; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; ID, iron deficiency; NYHA, New York Heart Association functional class; SBP, systolic blood pressure; Δ, change. Bold highlights the results for iron status—the focus of the article.

Relevance of iron deficiency in heart failure with preserved ejection fraction compared with heart failure with reduced ejection fraction sub-cohorts

Among patients with ID_{Ferritin}, fewer HFpEF patients died from any cause compared with HFrEF patients [39/258 (15%) vs. 140/642 (22%), respectively, $P = 0.03$].

There were 72 deaths and 170 combined deaths or rehospitalization in the HFpEF subgroup. There were more deaths when ID was defined by ID_{Tsat} 45/257 (18%) compared with ID_{Ferritin} 39/358 (15%) but the same number of deaths/HF hospitalization [105/257 (41%) vs. 105/258 (41%), respectively]. ID by either definition did not confer independent risk for either all-cause mortality [ID_{Ferritin} HR 0.65 (0.40–1.05),

Figure 1 Cumulative event curves for all-cause mortality in patients with ID_{Ferritin} and ID_{Tsats} compared with patients without ID during 2 years of follow-up. (A and C) Models adjusted for age/sex; (B and D) models adjusted for age, sex, ischaemic aetiology, New York Heart Association class, systolic blood pressure, atrial fibrillation, log(N-terminal pro-brain natriuretic peptide), and log(creatinine). ID, iron deficiency.

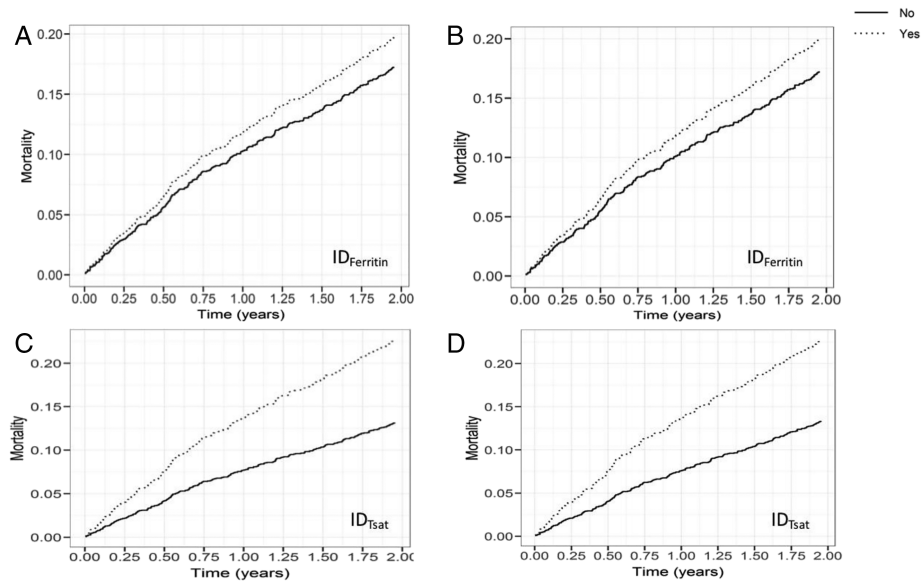
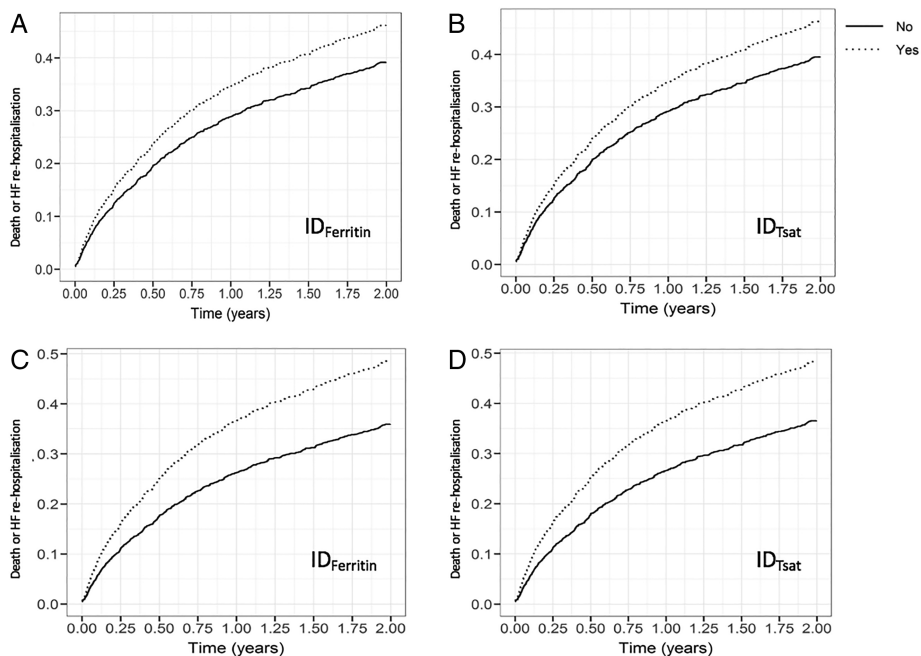


Figure 2 Cumulative event curves for death and HF hospitalization in patients with ID_{Ferritin} and ID_{Tsats} compared with patients without ID during 2 years of follow-up. (A and C) Adjusted for age/sex; (B and D) adjusted for age, sex, ischaemic aetiology, New York Heart Association class, systolic blood pressure, atrial fibrillation, log(N-terminal pro-brain natriuretic peptide), and log(creatinine). HF, heart failure; ID, iron deficiency.



$P = 0.08$; ID_{Tsats} HR 1.16 (0.72–1.87), $P = 0.55$) or death/HF hospitalization [ID_{Ferritin} HR 1.11 (0.81–1.52), $P = 0.52$; ID_{Tsats} HR 1.19 (0.69–1.84), $P = 0.28$] (Table 2). Absolute ID was also

not associated with increased rates of all-cause mortality [0.72 (0.44–1.19), $P = 0.20$] or death/HF hospitalization [1.17 (0.86–1.50), $P = 0.33$] (Supporting Information, Table S2).

Within the HF_{rEF} subgroup, there was a higher rate of all-cause mortality in those with ID_{Ferritin} compared with those without ID_{Ferritin} [140/642 (22%) vs. 81/473 (17%), respectively, $P = 0.063$] although the difference did not reach statistical significance. In Cox proportional hazard modelling adjusted for age and sex, ID_{Ferritin} was independently predictive of all-cause mortality [HR 1.41 (1.07–1.86), $P = 0.01$]. With adjustment for age, sex, ischaemic aetiology, NYHA class, SBP, AF, log(NT-proBNP), and log(creatinine), ID_{Ferritin} was no longer an independent predictor of all-cause mortality [HR 1.12 (0.85–1.48), $P = 0.43$] in patients with HF_{rEF}. In contrast, within HF_{rEF}, ID_{Tsat} was an independent risk factor for all-cause mortality in both models [adjusted for age and sex: HR 2.31 (1.71–3.11), $P < 0.01$; adjusted for age, sex, ischaemic aetiology, NYHA class, SBP, AF, log(NT-proBNP), and log(creatinine): HR 1.57 (1.15–2.14), $P < 0.01$]. For the combined endpoint of death or HF hospitalization in HF_{rEF} patients, ID_{Tsat} but not ID_{Ferritin} was an independent predictor of outcome [HR 1.24 (1.02–1.50), $P = 0.03$ vs. HR 1.11 (0.92–1.33), $P = 0.28$].

Relevance of change in iron status during the first 6 months

As we found ID defined by tsat criteria more clearly associated with outcomes, we focused on this definition for assessing the prognostic relevance of change in ID status. There were 96 (6%) deaths within the first 6 months of the study. Of these, 73 (76%) had ID_{Tsat} at baseline (in comparison, 61 were identified by ID_{Ferritin}). Of the 1467 patients who survived to 6 months, 988 had a blood sample available from a 6 month visit. Of these, 537 patients had ID_{Tsat} at baseline, of whom 284 (53%) had persistent ID_{Tsat} at 6 months. Of the 451 patients without ID_{Tsat} at baseline, 128 (20%) had developed ID_{Tsat} by their 6 month follow-up.

Patients with persistent ID_{Tsat} had an increased risk of all-cause mortality compared with those who never had ID_{Tsat} [HR 2.22 (1.42–3.46), $P < 0.01$] (Table 3, Figure 3). Persistent ID_{Tsat} remained a risk factor for all-cause mortality regardless of EF group [Model 2: HR 2.26 (1.45–3.53), $P < 0.01$], when adjusted for age and sex [Model 3: HR 2.34 (1.50–3.67), $P < 0.01$], change in NT-proBNP, and change in creatinine [Model 4: HR 2.16 (1.33–3.51), $P < 0.01$]. Persistent ID_{Tsat} was also an independent risk factor for death/HF hospitalization across all four models (Table 3, Figure 3).

Patients with baseline ID_{Tsat} resolved by 6 month follow-up had a similar risk of all-cause mortality as those who never had ID_{Tsat}. However, these patients did have an increased risk of the composite endpoint of death/HF hospitalization [HR 1.40 (1.06–1.86), $P = 0.02$] evident in all four models [Model 2: HR 1.38 (1.04–1.83), $P = 0.02$; Model 3: HR 1.43 (1.08–1.90), $P = 0.01$; Model 4: HR 1.46 (1.04–2.06), $P = 0.03$]. Patients who developed ID_{Tsat} during the first 6 months of

follow-up did not have a higher mortality rate or risk of death/HF hospitalization compared with those who never had ID_{Tsat}.

Discussion

This study demonstrated that ID was not predictive of either all-cause mortality or the combined endpoint of death/HF hospitalization in patients with HF_{pEF}, despite a similar prevalence of ID as patients with HF_{rEF}. Furthermore, the most widely studied definition of ID ('ferritin < 100 or 100–300 mcg/L with a tsat < 20%') was not an independent predictor of either mortality or the combined endpoint of death or HF admission in patients with HF_{rEF}. In contrast, ID defined as 'tsat < 20%' was independently predictive of both endpoints in patients with HF_{rEF}. Finally, this study demonstrated that the natural history of ID is important as persistence of ID_{Tsat} over 6 months was independently associated with poorer clinical outcomes than no ID, ID that resolves within 6 months, and newly developed ID.

Prevalence of iron deficiency in patients with heart failure with reduced ejection fraction and heart failure with preserved ejection fraction

The prevalence of ID in the current study is high (58%) consistent with previous reports.^{1,2,11–19} The prevalence of ID has been widely reported in patients with HF_{rEF} but not HF_{pEF}. A recent meta-analysis pooled the results of multiple small studies and found a prevalence for ID of 59% (95% CI 52–64%) in 1414 patients with HF_{pEF}.²⁰ The prevalence (58%) of ID in patients with HF_{pEF} in this study is consistent with this meta-analysis and with data from 448 HF_{pEF} patients is the largest single study of ID in patients with HF_{pEF} to date.

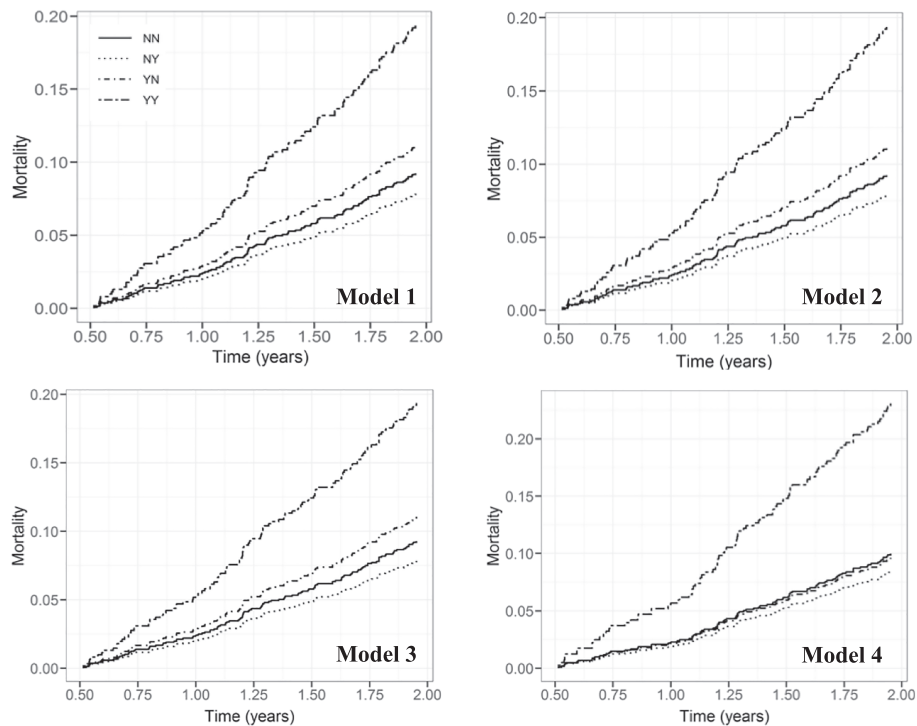
Association between heart failure with preserved ejection fraction, iron deficiency, and outcomes

No association between ID (by either definition) and all-cause mortality in patients with HF_{pEF} was found despite equally high prevalence of ID as in patients with HF_{rEF}. The overall mortality rate of 16% in the HF_{pEF} subgroup is relatively low but comparable with event rates in HF_{rEF} studies that have demonstrated an association between ID and mortality.^{3,5} The addition of HF hospitalizations to our secondary outcome did not improve the prognostic power of ID by either definition in the HF_{pEF} group. In comparison with patients with HF_{rEF}, those with the phenotype of HF_{pEF} did not have any distinguishing characteristics that would explain the difference in effect ID has on these two groups. Patients with HF_{pEF} were not included in the prior studies

Table 3 Association of change in ID_{Tsat} status over 6 months with outcome

	BL	6/12	Model 1 HR (95% CI)	Model 2 (Model 1 + EF) HR (95% CI)	Model 3 (Model 2 + age + gender) HR (95% CI)	Model 4 (age + gender + Δ logCreat + Δ Hb + Δ logNT-proBNP) HR (95% CI)
Hazard ratio for all-cause mortality						
ID [reference]	No	No				
ID	No	Yes	0.84 (0.41–1.72)	0.85 (0.42–1.74)	0.91 (0.44–1.86)	0.82 (0.37–1.83)
ID	Yes	No	1.21 (0.72–2.03)	1.20 (0.72–2.01)	1.38 (0.82–2.31)	0.99 (0.52–1.88)
ID	Yes	Yes	2.22 (1.42–3.46)	2.26 (1.45–3.53)	2.34 (1.50–3.67)	2.16 (1.33–3.51)
HF+EF				1.22 (0.82–1.81)	1.52 (0.99–2.31)	
Age (years)					1.05 (1.03–1.07)	1.06 (1.04–1.08)
Female					0.51 (0.32–0.82)	0.49 (0.30–0.81)
Δ Hb (g/dL)						1.01 (0.99–1.02)
Δ logCreatinine (μ mol/L)						1.74 (0.81–3.72)
Δ logNT-proBNP (pg/mL)						1.34 (1.14–1.57)
Hazard ratio for death or HF hospitalization						
ID [reference]	No	No				
ID	No	Yes	1.12 (0.78–1.61)	1.13 (0.79–1.62)	1.16 (0.81–1.66)	1.11 (0.73–1.69)
ID	Yes	No	1.4 (1.06–1.86)	1.38 (1.04–1.83)	1.43 (1.08–1.90)	1.46 (1.04–2.06)
ID	Yes	Yes	1.94 (1.49–2.51)	1.99 (1.53–2.59)	2.04 (1.57–2.65)	1.75 (1.30–2.37)
HF+EF				1.28 (1.02–1.61)	1.29 (1.01–1.64)	
Age (years)					1.01 (1.00–1.02)	1.01 (1.00–1.02)
Female					0.67 (0.52–0.86)	0.63 (0.47–0.84)
Δ Hb (g/dL)						1.01 (1.00–1.02)
Δ logCreatinine (μ mol/L)						2.21 (1.36–3.61)
Δ logNT-proBNP (pg/mL)						1.47 (1.34–1.62)

BL, baseline; CI, confidence interval; EF, ejection fraction; Hb, haemoglobin; HF, heart failure; HF+EF, heart failure with reduced ejection fraction; HR, hazard ratio; ID, iron deficiency; NT-proBNP, N-terminal pro-brain natriuretic peptide; Δ , change.
Reference group is NN (baseline no ID, 6/12 no ID).

Figure 3 Cumulative event curves for all-cause mortality by change in iron deficiency status over 6 months.

comparing circulating biomarkers of iron and ID definitions to BM iron stores⁷; thus, these definitions of ID may not be an accurate reflection of true ID in patients with HFpEF. Our results, although demanding independent corroboration, challenge any rationale for iron replacement in HFpEF at this time.

Importance of definition of iron deficiency on relationship between iron deficiency and mortality in patients with heart failure

It has been asserted that ID is an independent risk factor for mortality in HF despite published trials returning conflicting results.^{1–6} As with other populations suffering chronic disease, the definition of ID applied in the general population is ‘adjusted’ for the HF population to allow for a raised ferritin caused by inflammation. The contrary results may be explained by a lack of applicability for this ‘adjustment’ across the spectrum of HF. When our population of HF patients is compared with HF populations where a positive association was found, there are notable differences. For example, in the European Iron Consortium study of 1506 patients, ID was associated with an HR for mortality of 1.42 (95% CI 1.14–1.77, $P \leq 0.01$) on multivariable analysis (which included all univariate significant variables: age, sex, body mass index,

diabetes, NYHA functional class, LVEF, estimated glomerular filtration rate, high-sensitivity C-reactive protein, NT-proBNP, treatment with angiotensin-converting enzyme inhibitor and/or angiotensin receptor blocker, statins, loop diuretics, and the presence of anaemia or ID) compared with an HR of 1.00 (95% CI 0.78–1.27, $P = 0.97$) on multivariable analysis [age, sex, ischaemic aetiology, NYHA class, SBP, AF, log(NT-proBNP), and log(creatinine)] for ID in our study patients (Table 2).¹ The European population studied had more advanced HF than the current cohort, as reflected in higher NYHA class III/IV (61% vs. 29%) and 2 year mortality rates (29% vs. 19%). Of note, the European consortium cohort included a smaller proportion of patients with HFpEF (16% vs. 29%). A study of 574 patients with self-reported HF (EF unknown) in the USA found no association between ID and mortality.⁶ As in our study, the patients in this study had less advanced HF than the European cohort. In a study of 1684 patients (37% NYHA class III/IV), ID was found to be a predictor of mortality on univariate but not multivariable analysis incorporating adjustment for established predictors of mortality.⁴ The difference in results cannot be explained by inadequate event rates as our cohort and other negative studies have reported higher event rates than studies that have found a positive association between ID and mortality.² In our study, there was also no difference in baseline characteristics between those with ID_{Ferritin} and those with ID_{Tsat}.

Our study adds weight to the concern that ID_{Ferritin} , the most widely used definition of ID, does not best relate iron status to clinical outcomes across the spectrum of HF and in particular for patients with HFpEF. This may be because the ‘adjustment’ for inflammation is inaccurate. Definitions not requiring ferritin, or those using other inflammatory markers to guide the ‘adjustment’, may therefore be more suitable for HF patients who are less unwell (e.g. NYHA class I/II) or have HFpEF.

Until recently, the definitions of ID commonly used in patients with HF had not been correlated to BM iron stores, the gold standard definition of ID. Grote Beverborg *et al.* examined 42 patients undergoing coronary artery bypass graft with HF and $EF < 45\%$ and found that there was no strong correlation between ID defined as ID_{Ferritin} and BM iron stores (sensitivity 82.4%, specificity 75%). In contrast, a $tsat \leq 19.8\%$ strongly correlated with BM iron stores (sensitivity 94%, specificity 84%).⁷ This was subsequently validated in a cohort of 387 outpatients with known HFrEF.⁷ In a cohort of 1821 HFrEF patients, low $tsat (<20\%)$, but not low ferritin (<100 mcg/L), was associated with increased mortality.²¹ We found that $tsat < 20\%$ was a significant risk factor for both all-cause mortality and death or rehospitalizations in our 1563 patients. Our results further confirm $tsat < 20\%$ as the current definition of ID most closely related to adverse outcomes in HFrEF patients and therefore it should be considered as the preferred definition of ID in future trials assessing the effect of IV replacement therapy on morbidity and mortality in HFrEF.

Natural history of iron deficiency if left untreated

FAIR-HF and CONFIRM-HF established IV iron replacement therapy as effective at improving symptoms and reducing hospitalizations in ID_{Ferritin} patients with HFrEF, and a meta-analysis suggested IV replacement may reduce mortality.^{9,10,22} This has led to international guidelines recommending IV iron replacement in HF.^{23,24} However, the natural history of ID in the HF population and its relevance to outcome has not been previously examined. The current study has demonstrated that the natural history of ID is important. Persistent ID_{Tsats} is associated with a 60% higher mortality rate compared with maintaining normal iron homeostasis. However, patients with ID at baseline that resolved within 6 months did not have a higher risk of mortality on multivariable analysis. Curiously, patients who had ID at baseline had a higher risk of morbidity and mortality, but those who ‘developed ID’ in the first 6 months did not. We hypothesize several explanations for this. Firstly, we do not know how long patients with ‘developed ID’ were iron deficient. If they were ID for a similar duration as patients with baseline ID that ‘resolved’ by 6 months, then one would expect a similar lack of independent risk for mortality. However,

unlike patients with ‘resolved ID’, they did not have a higher risk of the composite endpoint of death/rehospitalization. This could be due to sample size as the number of patients who ‘developed ID’ was half the number with ‘resolved’ or ‘persistent’ ID or a difference in the severity of ID between these cohorts.

We did not find any baseline characteristics that could reliably predict those at risk of persistent ID despite patients with persistent ID being more likely to be co-morbid than the other groups, with a higher rate of AF, diabetes mellitus, and hypertension (Supporting Information, *Table S3*). They had equivalent use of anti-platelet, anti-coagulant, and oral iron supplementation. Haematinic variables were potentially more predictive. Hb, ferritin, iron, and transferrin saturation levels were all lower in those with persistent ID compared with other groups. In persistent ID, the median ferritin level was 90 vs. 125 mcg/L and mean Hb level 123 vs. 133 g/dL compared with resolved ID; however, the difference in $tsat$ was minimal (12% vs. 13%). Whether any cut-off values could be used to decide IV replacement therapy would require further study. Given the high rate of persistent ID (53%) and the association of persistent ID with mortality, we would suggest that the most pragmatic approach is to treat ID in HFrEF unless a clear clinical discriminator between those who will experience resolution versus persistence of ID can be established.

Limitations

This study was a sub-study of a prospective multicentre study that was powered for the primary outcome comparison of death from any cause between HFpEF and HFrEF. This sub-study did not have an independent power calculation specifically for the purposes of the ID analyses, and therefore, despite similar event rates to positive studies in HFrEF patients, we recognize it could be underpowered to detect a true association for ID_{Ferritin} and all-cause mortality or death/HF rehospitalization in patients with HFpEF. Not all patients could be included in this sub-study as some did not have serum samples available from their baseline visit for analysis. This may have introduced a selection bias; however, we did not detect an appreciable difference in baseline characteristics or event rates in our subgroup compared with the parent study. This study enrolled patients in both the outpatient and inpatient settings (immediately prior to discharge). We recognize this could be a confounding factor as there is a likelihood of an interaction between a patients’ acuity of disease and ferritin levels. However, we felt this was a strength from a practical perspective, as it would be easier for clinicians if one definition could accurately identify ID of prognostic importance in all stages of disease. Finally, whether IV iron

will be of benefit in patients with HFpEF can only be determined from prospective randomized controlled trials.

Conclusions

Iron deficiency is common among patients with HF with similar prevalence in patients with HFpEF and HFrEF. However, among patients with HFpEF, ID is not independently associated with adverse outcomes. This suggests that targeting this co-morbidity would not improve outcomes in this patient group. In contrast, ID is clearly associated with poor outcomes in HFrEF patients, particularly when it persists over time which occurs in more than half of ID patients if left untreated. In our patients with HFrEF, ID defined solely by 'tsat < 20%' was clearly a superior definition of ID than the more commonly used 'ferritin < 100 mcg/L or ferritin 100–300 mcg/L + tsat < 20%'. To optimize treatment efficacy, this definition should inform future trials investigating the role of IV iron replacement in patients with HFrEF. As patients with persistent ID have no distinguishing baseline characteristics to determine who is likely to self-resolve their ID, treatment with IV replacement should be given following initial diagnosis.

Conflict of interest

S.F. reports a grant from Green Lane Research and Educational Fund (NZ) during the conduct of the study. L.H.L. reports a grant from the National Medical Research Council of Singapore during the conduct of the study. K.P. reports grants from New Zealand Heart Foundation outside the submitted work. R.T. reports grants from Heart Foundation of NZ during the conduct of the study; grants and personal fees from Roche Diagnostics; and grants and personal fees from Merck, outside the submitted work. C.S.L. is supported by a Clinician Scientist Award from the National Medical Research Council of Singapore; has received research support from Boston Scientific, Bayer, Roche Diagnostics, Astra Zeneca, Medtronic, and Vifor Pharma; has served as consultant or on the Advisory Board/Steering Committee Executive Committee for

Boston Scientific, Bayer, Roche Diagnostics, AstraZeneca, Medtronic, Vifor Pharma, Novartis, Amgen, Merck, Janssen Research & Development LLC, Menarini, Boehringer Ingelheim, Novo Nordisk, Abbott Diagnostics, Corvia, Stealth BioTherapeutics, JanaCare, Biofourmis, Darma, Applied Therapeutics, MyoKardia, Cytokinetics, WebMD Global LLC, Radcliffe Group Ltd, and Corpus; and serves as co-founder and non-executive director of eKo.ai. A.M.R. reports grants from Health Research Council of New Zealand, grants from National Medical Research Council of Singapore, and other from Roche Diagnostics, during the conduct of the study; and grants, personal fees, non-financial support, and other from Roche Diagnostics, outside the submitted work. R.N.D. reports grants from NZ Health Research Council, grants from NZ Heart Foundation, and grants from Green Lane Research and Educational Fund (NZ) during the conduct of the study. T.J.Y., D.S., K.T.G.L., P.S.D.Y., H.Y.O., F.J., T.P.N., M.L., and G. D. have no competing interests.

Funding

This research was enabled by a grant from the Green Lane Research and Educational Fund (14/49/4107) for the iron assays in New Zealand. Funding sources and acknowledgements for the main study are included in the publication.⁸ R.N.D. is the recipient of the New Zealand Heart Foundation Chair of Heart Health. K.P. holds the New Zealand Heart Foundation Hynds Senior Fellowship.

Supporting information

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

Table S1. Baseline clinical characteristics ID_{Ferritin} vs ID_{Tsat}.

Table S2. Multivariable associations with outcome for absolute and functional ID.

Table S3. Baseline clinical characteristics by persistence or development of ID.

References

1. Klip IT, Comin-Colet J, Voors AA, Ponikowski P, Enjuanes C, Banasiak W, Lok DJ, Rosentryt P, Torrens A, Polonski L, van Veldhuisen DJ, van der Meer P, Jankowska EA. Iron deficiency in chronic heart failure: an international pooled analysis. *Am Heart J* 2013; **165**: 575–582.e3.
2. Jankowska EA, Rozentryt P, Witkowska A, Nowak J, Hartmann O, Ponikowska B, Borodulin-Nadziejka 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.
3. Okonko DO, Mandal AK, Missouri CG, Poole-Wilson PA. Disordered iron homeostasis in chronic heart failure: prevalence, predictors, and relation to anemia, exercise capacity, and survival. *J Am Coll Cardiol* 2011; **58**: 1241–1251.
4. Gonzalez-Costello J, Comin-Colet J, Lupon J, Enjuanes C, de Antonio M,

- Fuentes L, Moliner-Borja P, Farre N, Zamora E, Manito N, Pujol R, Bayes-Genis A. Importance of iron deficiency in patients with chronic heart failure as a predictor of mortality and hospitalizations: insights from an observational cohort study. *BMC Cardiovasc Disord* 2018; **18**: 206.
5. Nakano H, Nagai T, Sundaram V, Nakai M, Nishimura K, Honda Y, Honda S, Iwakami N, Sugano Y, Asaumi Y, Aiba T, Noguchi T, Kusano K, Yokoyama H, Ogawa H, Yasuda S, Chikamori T, Anzai T. Impact of iron deficiency on long-term clinical outcomes of hospitalized patients with heart failure. *Int J Cardiol* 2018; **261**: 114–118.
6. Parikh A, Natarajan S, Lipsitz SR, Katz SD. Iron deficiency in community-dwelling US adults with self-reported heart failure in the National Health and nutrition examination survey III: prevalence and associations with anemia and inflammation. *Circ Heart Fail* 2011; **4**: 599–606.
7. Grote Beverborg N, Klip IT, Meijers WC, Voors AA, Vegter 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.
8. Lam CSP, Gamble GD, Ling LH, Sim D, Leong KTG, Yeo PSD, Ong HY, Jaufeerally F, Ng TP, Cameron VA, Poppe K, Lund M, Devlin G, Troughton R, Richards AM, Doughty RN. Mortality associated with heart failure with preserved vs. reduced ejection fraction in a prospective international multi-ethnic cohort study. *Eur Heart J* 2018; **39**: 1770–1780.
9. Ponikowski P, van Veldhuisen DJ, Comin-Colet J, Ertl G, Komajda M, Mareev V, McDonagh T, Parkhomenko A, Tavazzi L, Levesque V, Mori C, Roubert B, Filippatos G, Ruschitzka F, Anker SD. 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.
10. Anker SD, Comin Colet J, Filippatos G, Willenheimer R, Dickstein K, Drexler H, Luscher TF, Bart B, Banasiak W, Niegowska J, Kirwan BA, Mori C, von Eisenhart RB, Pocock SJ, Poole-Wilson PA, Ponikowski P. Ferric carboxymaltose in patients with heart failure and iron deficiency. *N Engl J Med* 2009; **361**: 2436–2448.
11. Cohen-Solal A, Damy T, Terbah M, Kerebel S, Baguet JP, Hanon O, Zannad F, Laperche T, Leclercq C, Concas V, Duvillie L, Darne B, Anker S, Mebazaa A. High prevalence of iron deficiency in patients with acute decompensated heart failure. *Eur J Heart Fail* 2014; **16**: 984–991.
12. Yeo TJ, Yeo PS, Ching-Chiew Wong R, Ong HY, Leong KT, Jaufeerally F, Sim D, Santhanakrishnan R, Lim SL, Chan MMY, Chai P, Low AF, Ling LH, Ng TP, Richards AM, Lam CS. Iron deficiency in a multi-ethnic Asian population with and without heart failure: prevalence, clinical correlates, functional significance and prognosis. *Eur J Heart Fail* 2014; **16**: 1125–1132.
13. Rangel I, Goncalves A, de Sousa C, Leite S, Campelo M, Martins E, Amorim S, Moura B, Silva Cardoso J, Maciel MJ. Iron deficiency status irrespective of anemia: a predictor of unfavorable outcome in chronic heart failure patients. *Cardiology* 2014; **128**: 320–326.
14. Okonko DO, Grzeslo A, Witkowski T, Mandal AK, Slater RM, Roughton M, Foldes G, Thum T, Majda J, Banasiak W, Missouriis CG, Poole-Wilson PA, Anker SD, Ponikowski P. Effect of intravenous iron sucrose on exercise tolerance in anemic and nonanemic patients with symptomatic chronic heart failure and iron deficiency FERRIC-HF: a randomized, controlled, observer-blinded trial. *J Am Coll Cardiol* 2008; **51**: 103–112.
15. Schou M, Bosselmann H, Gaborit F, Iversen K, Goette JP, Soletomas G, Rasmussen J, Kistorp C, Kober L, Gustafsson F, Tonder N. Iron deficiency: prevalence and relation to cardiovascular biomarkers in heart failure outpatients. *Int J Cardiol* 2015; **195**: 143–148.
16. Makubi A, Hage C, Lwakatere J, Mbanda B, Kisenge P, Lund LH, Ryden L, Makani J. Prevalence and prognostic implications of anaemia and iron deficiency in tanzanian patients with heart failure. *Heart* 2015; **101**: 592–599.
17. Jankowska EA, Kasztura M, Sokolski M, Bronisz M, Nawrocka S, Oleskowska-Florek W, Zymlinski 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.
18. Jankowska EA, Malyszko J, Ardehali H, Koc-Zorawska E, Banasiak W, von Haehling S, Macdougall IC, Weiss G, McMurray JJ, Anker SD, Gheorghide M, Ponikowski P. Iron status in patients with chronic heart failure. *Eur Heart J* 2013; **34**: 827–834.
19. Ebner N, Jankowska EA, Ponikowski P, Lainscak M, Elsner S, Sliziuk V, Steinbeck L, Kube J, Bekfani T, Scherbakov N, Valentova M, Sandek A, Doehner W, Springer J, Anker SD, von Haehling S. The impact of iron deficiency and anaemia on exercise capacity and outcomes in patients with chronic heart failure. results from the studies investigating co-morbidities aggravating heart failure. *Int J Cardiol* 2016; **205**: 6–12.
20. Beale AL, Warren JL, Roberts N, Meyer P, Townsend NP, Kaye D. Iron deficiency in heart failure with preserved ejection fraction: a systematic review and meta-analysis. *Open Heart* 2019; **6**: e001012.
21. Moliner P, Jankowska EA, van Veldhuisen DJ, Farre N, Rozeny P, Enjuanes C, Polonski L, Merono 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.
22. Anker SD, Kirwan BA, van Veldhuisen DJ, Filippatos G, Comin-Colet J, Ruschitzka F, Luscher TF, Arutyunov GP, Motro M, Mori C, Roubert B, Pocock SJ, Ponikowski P. Effects of ferric carboxymaltose on hospitalisations and mortality rates in iron-deficient heart failure patients: an individual patient data meta-analysis. *Eur J Heart Fail* 2018; **20**: 125–133.
23. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten 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 J Heart Fail* 2016; **18**: 891–975.
24. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Colvin MM, Drazner MH, Filippatos GS, Fonarow GC, Givertz MM, Hollenberg SM, Lindenfeld J, Masouli FA, McBride PE, Peterson PN, Stevenson LW, Westlake C. 2017 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. *Circulation* 2017; **136**: e137–e161.