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ORIGINAL ARTICLE

Influence of serum transferrin concentration on diagnostic criteria for iron deficiency in chronic heart failure

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Abstract

Aims Transferrin saturation (TSAT), a marker of iron deficiency, reflects both serum concentrations of iron (SIC) and transferrin (STC). TSAT is susceptible to changes in each of these biomarkers. Little is known about determinants of STC and its influence on TSAT and mortality in patients with heart failure. Accordingly, we studied the relationship of STC to clinical characteristics, to markers of iron deficiency and inflammation and to mortality in chronic heart failure (CHF).

Methods and results Prospective cohort of patients with CHF attending a clinic serving a large local population. A total of 4422 patients were included (median age 75 (68–82) years; 40% women; 32% with left ventricular ejection fraction ≤40%). STC ≤ 2.3 g/L (lowest quartile) was associated with older age, lower SIC and haemoglobin and higher high-sensitivity C-reactive protein, ferritin and N-terminal pro-brain natriuretic peptide compared with those with STC > 2.3 g/L. In the lowest STC quartile, 624 (52%) patients had SIC ≤13 μmol/L, of whom 38% had TSAT ≥20%. For patients in the highest STC quartile, TSAT was <20% when SIC was >13 μmol/L in 185 (17%) patients. STC correlated inversely with ferritin ($r = -0.52$) and high-sensitivity C-reactive protein ($r = -0.17$) and directly with albumin ($r = 0.29$); all $P < 0.001$. In models adjusted for age, N-terminal pro-brain natriuretic peptide and haemoglobin, both higher SIC (hazard ratio 0.87 [95% CI: 0.81–0.95]) and STC (hazard ratio 0.82 [95% CI: 0.73–0.91]) were associated with lower mortality. SIC was more strongly associated with both anaemia and mortality than either STC or TSAT.

Conclusions Many patients with CHF and a low STC have low SIC even when TSAT is >20% and serum ferritin >100 μg/L; such patients have a high prevalence of anaemia and a poor prognosis and might have iron deficiency but are currently excluded from clinical trials of iron repletion.

Keywords Heart failure; Iron; Iron deficiency; Mortality; Transferrin saturation; Transferrin

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Introduction

For patients with chronic heart failure (CHF), timely identification and treatment of iron deficiency can improve symptoms and exercise capacity, reduce CHF-related hospitalizations and might reduce mortality.[1] How iron deficiency should be defined by blood markers in patients with CHF is uncertain. In clinical practice, serum ferritin is most commonly used, although diagnostic thresholds vary. Other blood markers (such as serum iron concentration and transferrin saturation [TSAT]) or more novel biomarkers (such as soluble transferrin receptor) may correlate better with bone marrow iron stores.[2,3]

Ferritin is almost exclusively intra-cellular, where it binds iron which would otherwise be highly cytotoxic. In health, only a small proportion of ferritin is shed into the circulation. The World Health Organization (WHO) suggests that a serum ferritin <15 μg/L is diagnostic of iron deficiency but inflammation and cell damage, characteristic of heart failure, may cause serum ferritin concentrations to rise even in the presence of iron

deficiency,[4,5] potentially leading to the diagnosis being missed. Attempting to account for this, guidelines for heart failure,[6,7] based on trial inclusion criteria,[8–11] provide a rather different definition of iron deficiency; a serum ferritin <100 μg/L or, if serum ferritin is 100–299 μg/L, a TSAT <20%. Importantly, TSAT is the ratio of the serum concentrations of iron (SIC) and transferrin (STC)[3,12] and is susceptible to variations in both SIC and STC. Transferrin, produced mainly in the liver, is the main molecule transporting iron in the blood. STC falls with inflammation and chronic disease,[12,13] and therefore, TSAT may be >20% even when SIC is low, masking a possible diagnosis of iron deficiency. When STC is high, TSAT may be <20% even when SIC is normal.

A TSAT <20%, irrespective of ferritin concentration, identifies patients with CHF at greater risk of heart failure hospitalization[14] and death.[3,15–17] However, previous research has shown that SIC is more strongly related to adverse outcomes than either TSAT or serum ferritin[18–20] and is strongly associated with 'gold-standard' bone marrow defined iron deficiency in patients with heart failure.[3] Therefore, there may be a group of patients at high risk of clinical events with low SIC but a normal TSAT, who are not currently considered iron deficient by guideline criteria.

Accordingly, we investigated the relationships between STC and patient characteristics, markers of iron deficiency, inflammation, and outcomes, in patients with CHF.

Methods

Study population

Between December 2001 and June 2019, consenting patients with suspected or confirmed heart failure referred for assessment at a community heart failure clinic serving a local population of about 550 000 people were enrolled in a registry conducted in parallel with routine NHS clinical activities, the Hull LifeLab. Blood samples were obtained on the same day as clinical examination, electro- and echocardiography, and included STC, SIC, serum ferritin and TSAT, calculated as (SIC (μmol/L)/STC (g/L) × 25.2) × 100.[3]

Heart failure was defined as typical symptoms and signs and either a measured or visually estimated left ventricular ejection fraction (LVEF) of ≤40% (HFrEF) or, if the LVEF was >40%, a raised N-terminal pro-brain natriuretic peptide (NT-proBNP) (≥ 125 ng/L).[21] Those with an elevated NT-proBNP without HFrEF were divided into those with an LVEF of 40–49% (heart failure with mid-range ejection fraction (HFmrEF)) or ≥50% (heart failure with preserved ejection fraction (HFpEF)). Some patients had a raised NT-proBNP but no available LVEF (n = 341) and were included in the analysis as HF-NT-proBNP. The WHO definition of anaemia was used (haemoglobin of <12.0 g/dL in women and <13.0 g/dL in men).[22] Only those with confirmed heart failure and complete haemoglobin and iron indices were included in this analysis. The population studied in this analysis is the same as in a previous publication.[20]

Two primary definitions of iron deficiency were used: SIC ≤ 13 μmol/L and TSAT < 20%.[3] The definition of iron deficiency currently adopted by guidelines (ferritin <100 μg/L or TSAT <20% if ferritin 100–299 μg/L)[6,7] was also used for some analyses.

Follow-up completed on the 3rd June 2019. Deaths were adjudicated based on available medical records: the adjudication process has been described previously.[23] The study was approved by the local research ethics committee.

Statistical analysis

Continuous variables are presented as medians with 25th and 75th percentiles and were compared using one-way ANOVA if normally distributed or by the Mann–Whitney U or Kruskal–Wallis test if not. Categorical variables, presented as numbers and percentages, were compared using chi-squared tests. Variables were transformed using either the square root or logarithms with base 10 as appropriate.[18] No imputation was performed for missing data. Patient characteristics are displayed and compared with the patients divided by quartiles of STC. Differences in STC between heart failure phenotypes were also examined. Univariable and multivariable linear regression analysis was used to identify correlates of STC. Variables associated with STC at the 10% significance level ($P \leq 0.1$) in univariable models were entered into the multivariable model. The strength and direction of effect between each variable and STC is reported using standardized beta coefficients and P values. We defined a low STC as ≤2.3 g/L; the lowest quartile. Characteristics of patients with a low STC were further classified by the presence or absence of a low SIC (≤13 μmol/L) and/or a low TSAT (<20%).

Kaplan–Meier survival curves for all-cause and cardiovascular (CV) mortality at 5 years were constructed by quartile of STC. Further survival curves were generated for a) those in the lowest quartile of STC by SIC (≤13 μmol/L or >13 μmol/L) and TSAT (<20% or ≥20%) and b) patients in each quartile of STC by SIC <10 μmol/L; 10–13 μmol/L; 14–17 μmol/L; >17 μmol/L. Heatmaps detailing all-cause mortality by STC relative to SIC were also constructed.

Cox proportional hazard regression models were used to assess the associations between clinical variables and all-cause and CV mortality within 5 years. A limited number of predictors for mortality, previously validated in two cohorts [age, blood urea, plasma NT-proBNP, haemoglobin, and prescription (or failure to prescribe) a beta-blocker],[24] were entered into multivariable models with the variables of interest and adjusted for SIC. Prescription of a loop diuretic was also added to multivariable models as it is a strong predictor of mortality.[18,25] Multivariable interaction analyses

were performed according to whether patients had HFrEF or not. If the analysis suggested that there was a significant interaction for a variable of interest, multivariable models were repeated separately for those with or without HFrEF.

All analyses were performed with SPSS statistical software version 27 (IBM). All tests were two-sided and unless previously specified, used a *P* value of <5% to determine statistical significance.

Results

Patient characteristics

Of 9321 patients enrolled between 1 December 2001 and June 2019, 7160 (77%) had a confirmed diagnosis of heart failure, of whom 4422 (62%) had full iron indices available for assessment. Those with heart failure and incomplete

Table 1 Characteristics according to quartiles of serum transferrin concentration (STC)

Variable	Q1 (≤2.3 g/L) (*n* = 1195; 27%)	Q2 (2.4–2.6 g/L) (*n* = 1159; 26%)	Q3 (2.7–2.9 g/L) (*n* = 1003; 23%)	Q4 (≥3.0 g/L) (*n* = 1065; 24%)	*P*-value
Demographics					
Age (years)	77 (70–83)	75 (67–82)	74 (67–80)	75 (67–81)	<0.001
Sex (female)	354 (30)	437 (38)	435 (43)	537 (50)	<0.001
Hypertension	637 (53)	640 (55)	564 (56)	614 (58)	0.21
Diabetes	284 (24)	285 (25)	280 (28)	303 (29)	0.02
IHD	537 (45)	477 (41)	421 (42)	441 (41)	0.22
COPD	120 (10)	102 (9)	82 (8)	107 (10)	0.35
History of cancer	59 (5)	34 (3)	24 (2)	31 (3)	0.004
Signs and symptoms					
NYHA III or IV	348 (30)	295 (26)	303 (31)	374 (36)	<0.001
ECG and ECHO					
AF/flutter	337 (29)	385 (34)	360 (38)	425 (41)	<0.001
Heart rate (b.p.m.)	71 (61–82)	73 (63–84)	74 (63–87)	76 (65–90)	<0.001
HFrEF	382 (32)	390 (34)	322 (32)	335 (32)	0.71
Bloods					
Haemoglobin (g/dL)	12.8 (11.5–14)	13.4 (12.2–14.5)	13.4 (12.2–14.6)	13.1 (11.9–14.4)	<0.001[a]
Anaemia	556 (47)	345 (30)	284 (28)	361 (34)	<0.001
MCV (fL)	90.7 (87.3–94.7)	90.3 (87.0–93.7)	89.8 (86.8–93.1)	88.2 (84.2–91.8)	<0.001[a]
MCH (pg)	30.0 (28.6–31.3)	29.8 (28.6–31.1)	29.8 (28.4–30.8)	29.0 (27.1–30.3)	<0.001[a]
MCHC (g/dL)	32.9 (32.1–33.8)	33.0 (32.2–33.8)	32.9 (32.2–33.7)	32.6 (31.6–33.5)	<0.001[a]
RDW (%)	14.2 (13.4–15.1)	14.0 (13.4–15.0)	14.2 (13.5–15.3)	14.6 (13.7–16.1)	<0.001
Iron (μmol/L)	13 (10–18)	15 (11–19)	15 (11–18)	13 (9–17)	<0.001
Iron ≤13 μmol/L	624 (52)	488 (42)	423 (42)	566 (53)	<0.001
TSAT (%)	25 (18–34)	23 (17–29)	21 (16–26)	16 (11–21)	<0.001
TSAT <20%	385 (32)	428 (37)	467 (47)	751 (71)	<0.001
TSAT <20% and iron >13 μmol/L	0 (0)	0 (0)	44 (4)	185 (17)	<0.001
Ferritin (μg/L)	147 (82–261)	95 (57–159)	73 (40–136)	41 (22–76)	<0.001
Ferritin <30 μg/L	20 (2)	72 (6)	145 (15)	391 (37)	<0.001
Ferritin <100 μg/L	393 (33)	609 (53)	628 (63)	876 (82)	<0.001
Guideline definition of iron deficiency	568 (48)	761 (66)	727 (73)	955 (90)	<0.001
eGFR (mL/min/1.73 m^2)	55 (38–71)	60 (46–75)	61 (48–77)	63 (49–77)	<0.001
hsCRP (mg/L)	5.4 (2.2–15.0)	3.9 (1.5–8.3)	3.5 (1.5–7.1)	3.8 (1.6–7.4)	<0.001
NT-proBNP (ng/L): SR	966 (417–2567)	724 (321–1745)	727 (288–1784)	600 (273–1829)	<0.001
NT-proBNP (ng/L): AF/Flutter	2334 (1309–4383)	1919 (1055–3331)	1824 (1073–3298)	1888 (1115–3190)	<0.001
Albumin (g/L)	36 (33–38)	38 (36–40)	38 (36–40)	38 (36–40)	<0.001
ALT (μ/L)	18 (14–24)	20 (15–26)	20 (16–26)	20 (16–26)	<0.001
ALP (μ/L)	77 (62–100)	76 (61–96)	74 (59–92)	80 (65–101)	<0.001
Medication					
Loop diuretic	755 (64)	702 (62)	647 (66)	721 (69)	0.005
ACEi or ARB	825 (70)	807 (71)	672 (68)	724 (69)	0.60
MRA	259 (22)	271 (24)	225 (23)	229 (22)	0.67
BB	747 (64)	708 (62)	609 (62)	664 (63)	0.83
Anticoagulants	305 (26)	353 (31)	315 (31)	365 (34)	<0.001
Antiplatelet agents	553 (46)	501 (43)	440 (44)	434 (41)	0.07
Oral iron	115 (9)	65 (6)	49 (5)	50 (5)	<0.001

ACEi, angiotensin converting enzyme inhibitor; AF, atrial fibrillation; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; BB, beta-blocker; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; ESC, European Society of Cardiology; HFrEF, heart failure with reduced ejection fraction; hsCRP, high-sensitivity CRP; ID, iron deficiency; IHD, ischaemic heart disease; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N terminal pro-natriuretic peptide; NYHA, New York Heart Association; RDW, red cell distribution width; SR, sinus rhythm; TSAT, transferrin saturation.
[a]Variables normally distributed and compared using one-way ANOVA.

haemoglobin or iron indices not included in this analysis (n = 2738; 38%) were more likely to be enrolled prior to 2009, were younger and were more likely to have HFrEF than those with complete results but there was no difference in sex distribution or plasma concentrations of NT-proBNP (*Table S1*). More than half of those included had either HFmrEF (19%) or HFpEF (41%) while 32% had HFrEF. A few (n = 341; 8%) had echocardiographic data but no record of LVEF (HF-NT-proBNP). Differences in clinical characteristics between patients of various heart failure phenotypes have previously been published.[20]

Patients in the lowest STC quartile [≤2.3 g/L: n = 1195 (27%)] were older, more likely to be men, to have a previous or prevalent diagnosis of cancer and to have anaemia. They had lower haemoglobin, estimated glomerular filtration rate (eGFR) and albumin and higher serum ferritin, TSAT, high-sensitivity C-reactive protein (hsCRP) and plasma NT-proBNP (*Tables 1* and *S2*). The prevalence of iron deficiency by the ESC criteria was lower in those with low STC. STC was similar amongst heart failure phenotypes (*Figure S1*).

For patients with a low STC, just over half had a low SIC (≤13 μmol/L) (*Figure 1*). Compared with those with a low STC and normal SIC (>13 μmol/L), those with both a low STC and SIC were older, more likely to be anaemic, more likely to be women and had more severe symptoms of CHF with higher plasma NT-proBNP (*Table S3*). In the absence of a low SIC, no patient had a TSAT <20%. Of those with a low SIC, more than a third (38%, n = 239) had a TSAT ≥20% because STC was low. These patients, despite a high prevalence of anaemia and low SIC, would not be considered iron deficient by current ESC criteria unless ferritin was also low.[21] Characteristics of patients with a low SIC with a TSAT above or below 20% are shown in *Table S4*. Of patients in the highest quartile of STC (n = 1065), 17% (n = 185) had a normal SIC (>13 μmol/L) but a TSAT <20% because STC was high.

Variables associated with serum transferrin

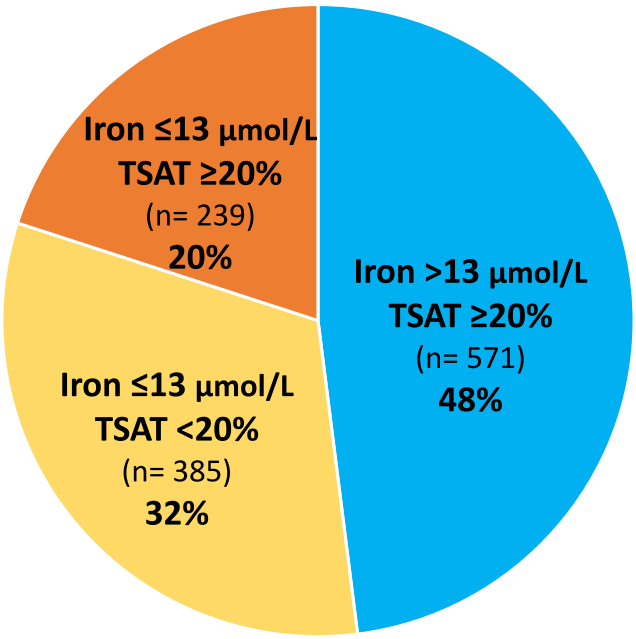
In univariate analysis there was an inverse relation between STC and serum ferritin and much weaker inverse relations with SIC, NT-proBNP, age, and hsCRP. There were positive correlations between STC and haemoglobin, eGFR, and serum albumin (*Figure S2*).

In multivariable analysis, there were inverse associations between STC and age (r = −0.11; P < 0.001) and serum ferritin (r = −0.52; P < 0.001) and a positive correlation with serum albumin (r = 0.29; P < 0.001) (*Table S5*). In the model, NT-proBNP interacted with eGFR, albumin, and hsCRP. Despite repeating the multivariable model with these variables removed, there was no correlation between NT-proBNP and STC.

Survival

Median follow-up was 49 (18–89) months, during which 2321 (52.5%) patients died; 5 year mortality was 34.5% (n = 1526).

Figure 1 Measures of iron status in patients in the lowest quartile (≤2.3 g/L) of serum transferrin concentration (STC). Serum iron concentration (SIC) in μmol/L.



In univariate analysis, STC, as a continuous variable, was inversely associated with all-cause mortality (HR [95% CI]: HR 0.75 [0.67–0.83] per 1 g/dL; $P < 0.001$) (*Table S6*). Compared with those with an STC > 2.3 g/dL, those in the lowest quartile had a higher all-cause (HR 1.41 [1.27–1.57]; $P < 0.001$), cardiovascular (HR 1.31 [1.13–1.52]; $P < 0.001$) and non-cardiovascular (HR 1.57 [1.34–1.84]; $P < 0.001$) mortality (*Figures 2A* and *S3a*). A greater proportion of deaths in the lowest quartile of STC was due to cancer, with lung cancer being most common (*Table 2*).

Figure 2 (A) Kaplan–Meier survival curves for all-cause mortality in all patients (n = 4422) by quartiles of serum transferrin concentration (STC). Log-rank P-value provided. Unadjusted hazard ratios and 95% confidence intervals for each quartile of STC with quartile two (2.4–2.6 g/L) as reference.(B) Kaplan–Meier survival curves for all-cause mortality for patients in the lowest quartile (≤2.3 g/L) of serum transferrin concentration (STC) according to serum iron concentration (SIC) above or below 13 μmol/L or TSAT above or below 20%. Log-rank P-values provided. Unadjusted hazard ratios and 95% confidence intervals for each group (SIC >13 μmol/L + TSAT ≥20% (reference group); SIC ≤13 μmol/L + TSAT ≥20%; SIC ≤13 μmol/L + TSAT <20%). No patient had SIC >13 μmol/L + TSAT <20%.

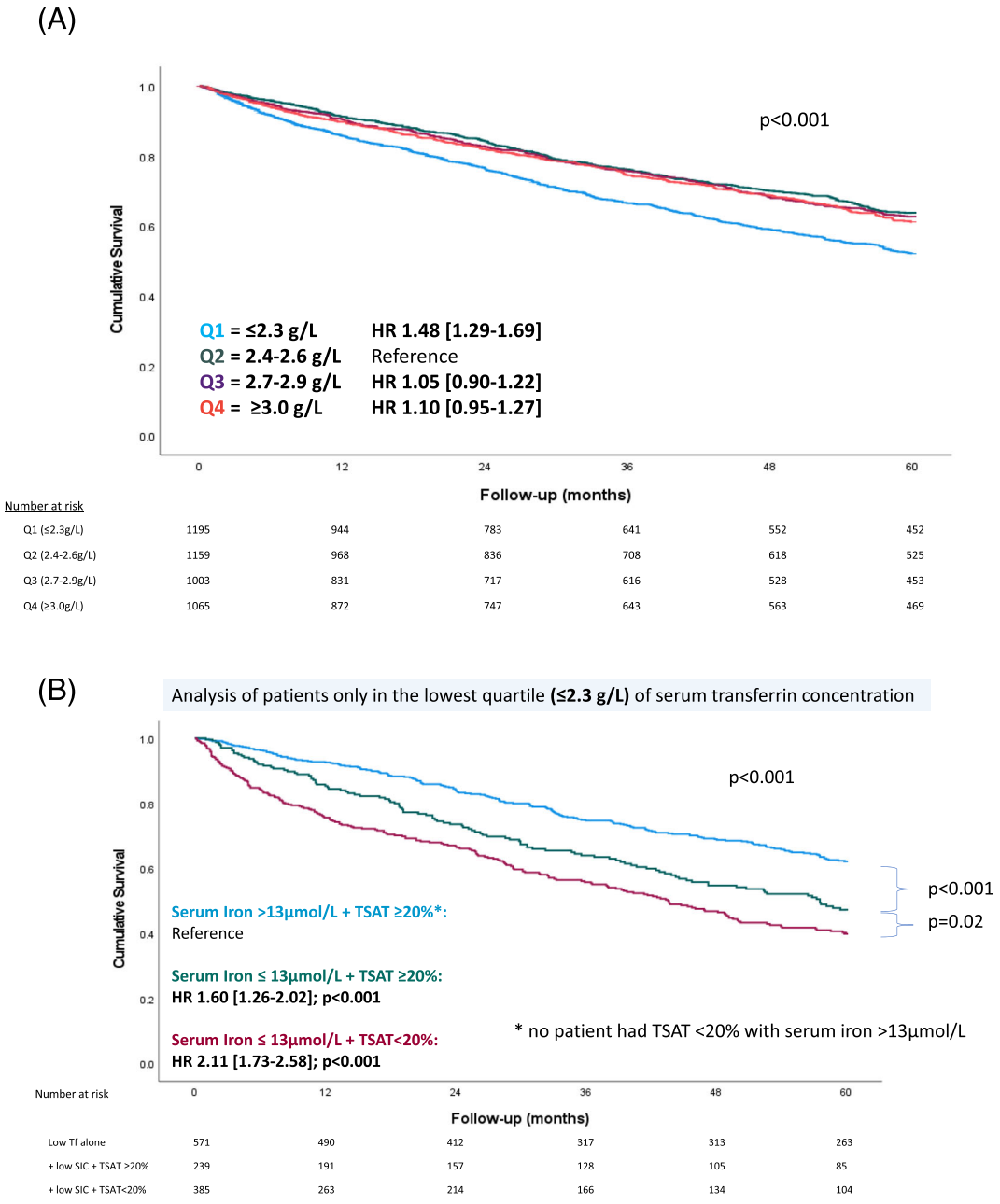


Table 2 Deaths in participants by quartiles of serum transferrin concentration (STC)

	Q1 (≤2.3 g/L) (*n* = 1195; 27%)	Q2 (2.4–2.6 g/L) (*n* = 1159; 26%)	Q3 (2.7–2.9 g/L) (*n* = 1003; 23%)	Q4 (≥3.0 g/L) (*n* = 1065; 24%)	*P*-value
Deaths (1 year)	155 (14%)	91 (9%)	87 (10%)	103 (11%)	<0.001
CV death (as % of deaths)	87 (56%)	54 (59%)	52 (60%)	69 (67%)	0.38
Non-CV deaths (as % of deaths)	64 (41%)	35 (39%)	32 (37%)	32 (31%)	0.42
Unknown cause (as % of deaths)	4 (3%)	2 (2%)	3 (3%)	2 (2%)	N/A
Cancer deaths (as % of deaths)	27 (17%)	11 (12%)	5 (6%)	10 (10%)	0.05
Deaths (2 years)	243 (24%)	154 (16%)	144 (17%)	167 (18%)	<0.001
CV deaths (as % of deaths)	133 (55%)	91 (59%)	80 (56%)	113 (68%)	0.05
Non-CV deaths (as % of deaths)	104 (43%)	59 (38%)	63 (44%)	53 (32%)	0.09
Unknown cause (as % of deaths)	6 (2%)	4 (3%)	1 (<1%)	1 (<1%)	N/A
Cancer deaths (as % of deaths)	42 (17%)	23 (15%)	15 (10%)	16 (10%)	0.09
Deaths (5 years)	497 (42%)	356 (31%)	321 (32%)	352 (33%)	<0.001
CV death (as % of deaths)	252 (51%)	193 (54%)	170 (53%)	199 (57%)	0.39
Non-CV death (as % of deaths)	233 (47%)	152 (43%)	140 (44%)	144 (41%)	0.35
Unknown cause (as % of deaths)	12 (2%)	11 (3%)	11 (3%)	9 (2%)	N/A
Cancer deaths (as % of deaths)	79 (16%)	51 (14%)	36 (11%)	35 (10%)	0.048
Types of cancer deaths at 5 years (as % of cancer deaths)					
Upper GI	4 (5%)	2 (4%)	4 (11%)	2 (6%)	0.53
Lower GI	4 (5%)	3 (6%)	2 (6%)	5 (14%)	0.32
Lung	25 (32%)	14 (28%)	9 (25%)	14 (40%)	0.52
Breast	2 (3%)	3 (6%)	5 (14%)	2 (6%)	0.13
Prostate	10 (13%)	4 (8%)	1 (3%)	0 (0%)	0.07
Other solid organ	15 (19%)	8 (16%)	8 (22%)	6 (17%)	0.88
Haematological	13 (16%)	9 (17%)	5 (14%)	3 (9%)	0.66
Unknown	6 (8%)	8 (16%)	2 (6%)	3 (9%)	0.35

CV, cardiovascular; GI, gastrointestinal.

A low SIC was associated with greater all-cause mortality (*Table S6*) regardless of STC (*Figure S4*), but prognosis was worst in those with both low SIC and STC (*Figures 3* and *4*).

In analyses restricted to those with a low STC, patients with low SIC had higher all-cause and CV mortality than those with a normal SIC (*Figures 2B* and *S3b*). Examining outcomes by the lowest two (Q1 and Q2) and highest two quartiles (Q3 and Q4) of STC, a low SIC identified those at higher risk of death better than a low TSAT, irrespective of STC (*Figure 5*).

Overall, in multivariable analysis, STC as a continuous variable was inversely related to all-cause mortality (HR 0.82

Figure 3 Heat maps detailing all-cause mortality within 5 years by STC and SIC. Number of patients (A) and proportion (%) of patients with TSAT <20% and median and (Q1–Q3) TSAT (B) shown. Red indicates higher and blue lower risk. For instance, there were 298 patients with a STC ≤2.3g/L and SIC ≤10μmol/L, 98% of whom had a TSAT <20%, median TSAT 14 (12-16)%. This was the highest risk subset in the cohort.

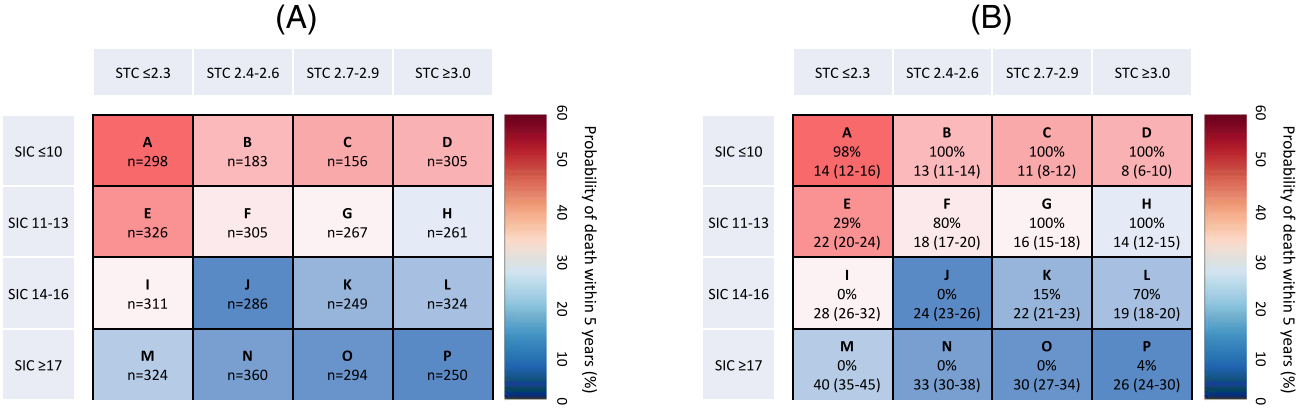


Figure 4 Diagram detailing number of patients, prevalence of anaemia, TSAT <20% and median (Q1–Q3) serum ferritin with mortality within 5 years displayed as Hazard Ratios and (95% confidence intervals) according to each quartile of serum transferrin concentration (STC) if serum iron concentration (SIC) was low (≤13 μmol/L) or normal (>13 μmol/L). Mortality analysis (all-cause mortality) adjusted for age, NT-proBNP, urea, haemoglobin, treatment with beta-blockers, and loop diuretics.

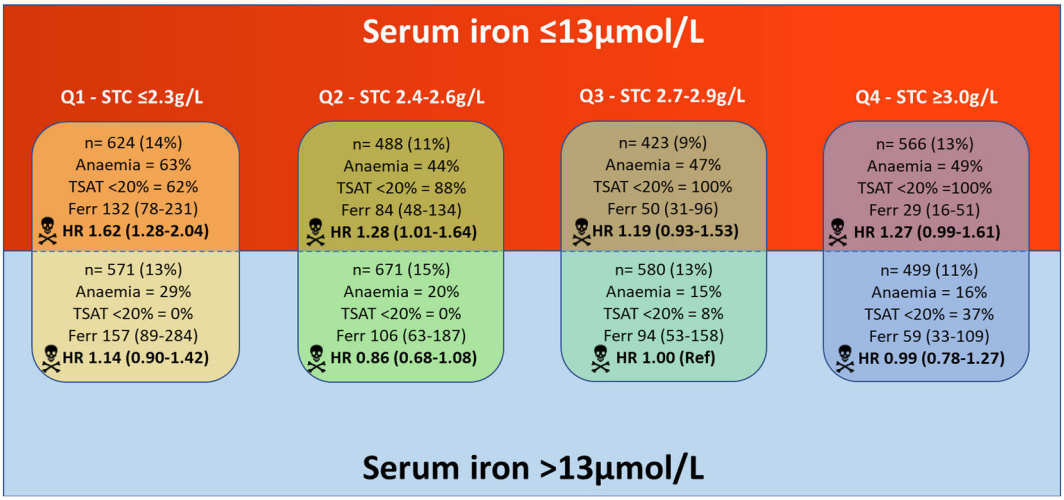
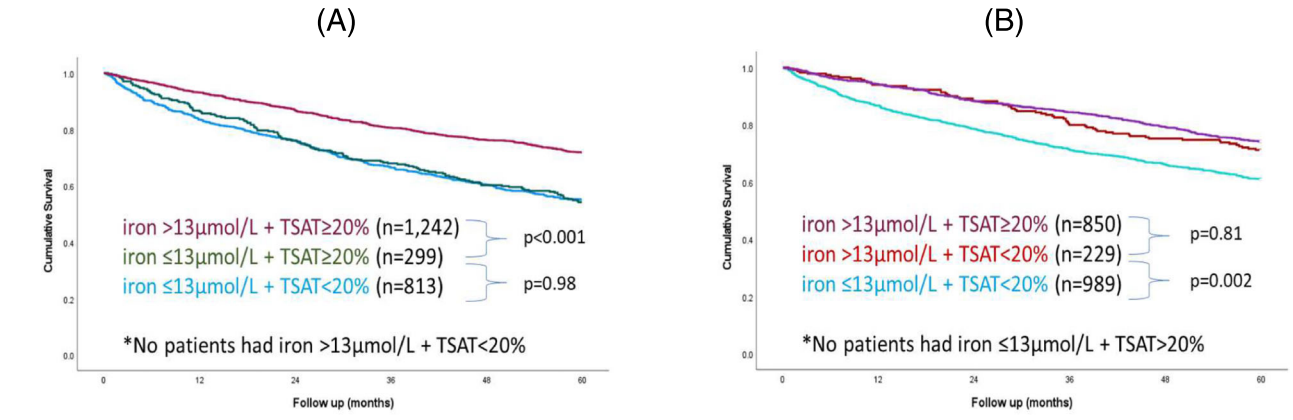


Figure 5 Kaplan–Meier survival curves for all-cause mortality for patients within quartiles 1 and 2 (A) and quartiles 3 and 4 (B) according to serum iron concentrations (≤13 μmol/L or >13 μmol/L) and TSAT (<20% or ≥20%). Log-rank *P*-values provided. Serum iron >13 μmol/L and TSAT ≥20% used as reference in each. *P*-values between groups also provided. No patient had SIC >13 μmol/L + TSAT <20%.



[0.73–0.91] $P < 0.001$) (*Table 3*). STC in the lowest quartile was also associated with higher all-cause mortality (HR 1.24 [1.11–1.39]; $P < 0.001$). However, in multivariable analyses, neither STC as a continuous variable nor the lowest quartile of STC as a categorical variable were associated with CV mortality. Analysis by quartiles of STC showed interactions by CHF phenotype for all-cause mortality ($P = 0.008$). In patients with an LVEF >40%, the lowest quartile of STC was associated with higher all-cause mortality (HR 1.24 [1.11–1.39]; $P < 0.001$). Repeating the analysis with STC as a continuous variable did not confirm significant interactions between phenotype and all-cause mortality ($P = 0.27$).

Table 3 Multivariable Cox regression analysis of all-cause and cardiovascular mortality

	All-cause mortality		Cardiovascular mortality	
Variable	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (/10 years)	1.44 (1.35–1.53)	<0.001	1.33 (1.23–1.45)	<0.001
Urea (/5 mmol/L)	1.16 (1.11–1.21)	<0.001	1.16 (1.10–1.23)	<0.001
NT-proBNP (Log10 ng/L)	1.97 (1.76–2.21)	<0.001	2.73 (2.33–3.20)	<0.001
Haemoglobin (g/dL)	0.93 (0.89–0.96)	<0.001	0.92 (0.87–0.96)	<0.001
Beta-blocker (yes/no)	0.78 (0.70–0.87)	<0.001	0.76 (0.65–0.88)	<0.001
Loop diuretic (yes/no)	1.44 (1.26–1.63)	<0.001	1.47 (1.23–1.77)	<0.001
SIC (sq. root 1 μmol/L)	0.87 (0.81–0.95)	<0.001	1.02 (0.91–1.13)	0.75
STC (1 g/L)	0.82 (0.73–0.91)	<0.001	0.95 (0.82–1.10)	0.51
The following was added to the above model [excluding serum transferrin concentration (STC) as a continuous measure]				
STC ≤ 2.3 g/L*	1.24 (1.11–1.39)	<0.001	1.15 (0.98–1.34)	0.09
The following continuous variables were added individually to the above model (excluding serum iron concentration (SIC) as a continuous variable)				
TSAT (Log10) (%)	0.61 (0.45–0.83)	0.001	1.08 (0.70–1.65)	0.73
Ferritin (Log 10) (1 μg/L)	1.19 (1.01–1.40)	0.03	1.45 (1.16–1.81)	0.001
The following categorical variables were added individually to the above model [excluding serum iron concentration (SIC) as a continuous variable]				
SIC ≤13 μmol/L	1.30 (1.15–1.46)	<0.001	1.04 (0.88–1.22)	0.67
TSAT <20%	1.26 (1.12–1.42)	<0.001	0.99 (0.84–1.17)	0.93
Ferritin <30 μg/L	1.03 (0.87–1.22)	0.71	0.90 (0.71–1.15)	0.41
Ferritin <100 μg/L	0.95 (0.84–1.07)	0.37	0.79 (0.67–0.93)	0.004
Guideline definition of iron deficiency	1.06 (0.94–1.20)	0.35	0.93 (0.79–1.11)	0.43

ESC, European Society of Cardiology; ID, iron deficiency; NT-proBNP, N terminal pro-natriuretic peptide; TSAT, transferrin saturation.

Discussion

We have identified patients with CHF and a low SIC (≤13 μmol/L), low haemoglobin and a high mortality who do not fulfil the current guideline definition of iron deficiency and have been excluded from landmark trials of iron repletion.[6,26] We have also identified patients with a normal SIC who do fulfil the current guideline definition of iron deficiency, who have a relatively low prevalence of anaemia and relatively good prognosis who may not have iron deficiency; their inclusion in randomized trials of iron repletion for heart failure may dilute any observed benefits.

In health, a low STC is associated with being iron replete.[27,28] By contrast, in patients with CHF, a low STC is associated with a higher hsCRP, suggesting that inflammation may be an important determinant of STC. Importantly, TSAT is a ratio. When STC is low, TSAT may be normal (≥20%) despite a low SIC. Amongst patients in the lowest quartile of STC, 38% of those with a TSAT ≥20% also had low SIC. When STC is high, even when SIC is normal, TSAT may be low: 71% of patients in the highest STC quartile had a TSAT <20% but 25% of these had normal SIC.

Bone-marrow histology is the gold-standard method for diagnosing iron deficiency. SIC ≤13 μmol/L more accurately predicts bone marrow iron depletion than does the current definition of iron deficiency in heart failure guidelines.[3] Serum iron is also a better predictor of mortality than either guideline defined iron deficiency or a TSAT <20%,[19] possibly because it is a better marker of iron deficiency. We have also shown that resolution of low SIC is associated with better survival but resolution of iron deficiency defined by guideline criteria or by a TSAT <20% is not.[19]

We found inverse correlations between STC and both serum ferritin and hsCRP. This is consistent with the hypothesis that inflammation and/or cell damage might increase release of ferritin from cells[4,5,13] but suppress the production of STC. When inflammatory pathways are activated, serum ferritin may be high even in the context of severe iron deficiency.[3] We now show that inflammation is also associated with a fall in STC that may normalize TSAT in the presence of a low SIC. Regardless of heart failure phenotype, lower STC was associated with a higher mortality independent of SIC. Patients with a lower STC had several features associated with worse prognosis, including being older and having higher plasma NT-proBNP, as well as a higher hsCRP.

Patients with a low STC were at higher risk of both cardiovascular and non-cardiovascular deaths, including cancer. This was especially the case for HFmrEF/HFpEF, for whom cancer accounts for a larger proportion of deaths than for HFrEF.[29] This may help explain why a STC ≤ 2.3 g/L was most clearly associated with a higher all-cause mortality in patients with HFmrEF/HFpEF. Cancer is known to be associated with a fall in STC[28,30,31] possibly due to upregulation and over-expression of transferrin receptors on cancer cells, leading to internalization of transferrin/transferrin-receptor complexes.[32]

The results of clinical trials for any intervention reflect the net effects of the benefits and harms that it may bring about; when benefit outweighs harm, then the trial may have a positive result. This does not mean that all patients who are

included in a positive trial benefited from the intervention. The current guideline definition of iron deficiency is neither an accurate marker of bone marrow iron depletion nor strong predictor of prognosis.[3,19] If the definition of iron deficiency includes many patients who are iron replete, then it will dilute the apparent benefits of iron replacement therapy in clinical trials and expose patients to unnecessary treatment in clinical practice. Furthermore, it is possible that administration of intravenous iron to patients who are already iron replete could be harmful. Post–hoc analyses of ongoing clinical trials may provide some answers. On the other hand, many patients with evidence of iron deficiency by other criteria, including a low SIC and a low STC, were excluded from randomized trials of iron therapy. Consequently, it is unclear if such patients benefit from iron repletion. Administration of intravenous iron to patients with iron deficiency and a low STC is probably safe. Current formulations release only small amounts of unbound iron into the circulation before uptake by the reticuloendothelial system.[33] The rate of subsequent release of iron depends on the availability of unsaturated transferrin rather than the dose of IV iron or the rate of infusion.[34]

Limitations

Participants were from a single clinic and were mainly Caucasian; results might not apply to other ethnic groups. Patients were recruited over a period of nearly 20 years during which time, treatment for CHF has improved. Novel biomarkers of iron status, such as soluble transferrin receptor, were not available to us. A proportion of those with confirmed heart failure (32%) did not have full iron indices or haemoglobin available and were therefore not included in this analysis. We did not do bone marrow biopsies to confirm the presence of iron deficiency. It is possible that serum iron biomarkers are associated with mortality by pathways other than their association with iron deficiency, such as inflammation.

Conclusions

Many patients with CHF and a low STC have a low SIC even when TSAT is ≥20% and serum ferritin >100 μg/L; such patients have a high prevalence of anaemia and a poor prognosis and might have iron deficiency but are currently excluded from clinical trials of iron repletion. Many patients with CHF and a high STC have a TSAT <20% but have a normal SIC, have a relatively low prevalence of anaemia, and have a better prognosis; if such patients are not truly iron deficient, then their inclusion in clinical trials of iron repletion may dilute observed benefits. More accurate definitions of iron deficiency should increase the benefits of iron repletion in both clinical trials and in clinical practice.

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Conflict of interest

F.J.G. reports receipt of sponsorship from Pharmacosmos to attend an international meeting. P.P. reports consulting fees from Vifor and Pharmacosmos. J.G.F.C. reports receipt of personal honoraria for lectures and advisory boards from Pharmacosmos and Vifor, and from AstraZeneca, Amgen, Bayer, Novartis and Servier. The University of Glasgow has received research grants from Pharmacosmos and Vifor. The rest of the authors confirm no conflict of interest.

Supporting information

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

Figure S1. Bar graphs detailing median transferrin and % of patients with a low transferrin (≤2.3 g/L) according to heart failure phenotypes.
Figure S2. Scatterplots demonstrating the correlation between transferrin (x-axis) and various biomarkers (y-axis). Corresponding correlation coefficients and *p*-values are provided for each variable.
Figure S3a. Kaplan–Meier survival curves for cardiovascular mortality in all patients (n = 4422) by quartiles of serum transferrin. Log-rank *p*-value provided. Hazard ratios and 95% confidence intervals for each quartile of transferrin provided with quartile two (2.4–2.6 g/L) as reference.
Figure S3b. Kaplan–Meier survival curves for cardiovascular mortality in those with a transferrin (Tf) ≤ 2.3 g/L (n = 1195) according to serum iron and TSAT. Log-rank *p*-values provided. Hazard ratios and 95% confidence intervals for each group with low transferrin alone with normal serum iron and TSAT used as the reference group.
Figure S4a. Kaplan–Meier survival curves for all-cause mortality by serum iron concentration for patients in the 1st (n = 1195) quartile of serum transferrin. Log-rank *p*-value provided. Hazard ratios and 95% confidence intervals for each level of serum iron provided. Serum iron >17 μmol/L used as the reference in each.
Figure S4b. Kaplan–Meier survival curves for all-cause mortality by serum iron concentration for patients in the 2nd

(n = 1159) quartile of serum transferrin. Log-rank *p*-value provided. Hazard ratios and 95% confidence intervals for each level of serum iron provided. Serum iron >17 μmol/L used as the reference in each.

Figure S4c. Kaplan–Meier survival curves for all-cause mortality by serum iron concentration for patients in the 3rd (n = 1003) quartile of serum transferrin. Log-rank *p*-value provided. Hazard ratios and 95% confidence intervals for each level of serum iron provided. Serum iron >17 μmol/L used as the reference in each.

Figure S4d. Kaplan–Meier survival curves for all-cause mortality by serum iron concentration for patients in the 4th (n = 1065) quartile. Log rank *p*-value provided. Hazard ratios and 95% confidence intervals for each level of serum iron provided. Serum iron >17 μmol/L used as the reference in each.

Table S1. Key characteristics of those with heart failure and all iron and haemoglobin results available vs those with missing tests.

Table S2. Characteristics according to serum transferrin concentration.

Table S3. Characteristics of patients with serum transferrin in the lowest quartile (n = 1195) according to serum iron values.

Table S4. Characteristics of patients with the lowest quartile of transferrin (n = 1195) according to serum iron and TSAT (there were no patients with serum iron >13 μmol/L).

Table S5. Univariate and multi-variable associations with serum transferrin.

Table S6. Univariable Cox regression analysis for all-cause and cardiovascular mortality.

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