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RESEARCH ARTICLE

Iron deficiency and red cell indices in patients with heart failure

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Aims To investigate the prevalence of iron deficiency (ID) in heart failure (HF) patients with normal vs. abnormal red cell indices (RCI), the associations between iron parameters and RCI, and prognostic consequences of ID independently of RCI.

Methods and results We analysed clinical data of 1821 patients with HF [mean age 66 ± 13 years; 71% men; New York Heart Association class I/II/III/IV (11%/39%/44%/6%); left ventricular ejection fraction >45%: 19%]. Iron deficiency (ferritin <100 μg/L or ferritin 100–299 μg/L with transferrin saturation <20%) was common irrespective of the presence of anaemia (haemoglobin <12 g/dL in women and <13 g/dL in men) or low RCI, from 75% in anaemic subjects with low mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and MCH concentration (MCHC), to 36% in non-anaemic subjects with MCV, MCH, and MCHC above the lower limit of normal. After adjustment for clinical variables, iron parameters remained independently associated with haemoglobin, MCV, MCH, MCHC, mean reticulocyte haemoglobin content (CHR), and red cell distribution width (RDW). In multivariable Cox proportional hazard regression models there was a trend towards higher mortality in patients with vs. without ID when adjusted for relevant HF prognosticators and MCH or MCHC (but not haemoglobin, CHR or RDW).

Conclusions Patients with HF should be routinely screened for ID irrespective of the presence of anaemia or abnormal RCI. The detrimental impact of ID on long-term survival in HF is partially independent of RCI.

Keywords Heart failure • Iron deficiency • Anaemia • Red cell indices • Complete blood count

Introduction

In recent years much attention has been paid to disordered iron status and its adverse consequences for the symptomatology and prognosis in heart failure (HF).[1–4] The prevalence of iron deficiency (ID) in chronic HF patients ranges from 50% in Europe[5] to 61% in a multi-ethnic Asian population.[6] Iron deficiency predicts decreased exercise capacity,[7] worse prognosis,[4,5,8] and, importantly, appears to be a promising therapeutic target.[9–13] Given the importance of sufficient availability of iron for unrestricted erythropoiesis within the bone marrow,[14,15] ID has been traditionally perceived as an aetiological factor of anaemia.[16] Indeed, in daily clinical practice haemoglobin concentration and automatically measured red cell indices (RCI) are considered sensitive indicators of systemic iron status,[14,17] and HF patients without anaemia and with normal RCI are rarely screened for ID. We compared the prevalence of ID in HF patients with normal and abnormal RCI, and assessed the associations between different iron parameters and

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particular RCI. Furthermore, we evaluated the prognostic consequences of ID independently of abnormal RCI or anaemia.

Methods

Patients

The study population comprised 1821 patients with chronic HF, from five cohorts from: (i) Poland (two cohorts, $n = 735$),[7,8] (ii) Spain (one cohort, $n = 789$),[18] and (iii) the Netherlands (two cohorts, $n = 297$),[19,20] as previously described by Klip *et al.*[5] Detailed information on inclusion and exclusion criteria for each cohort are available online in the appendix to aforementioned paper.[5] No patient received blood transfusions, erythropoietin therapy, or intravenous iron therapy at the time of inclusion. All study protocols have been approved by the local ethics committees, and all patients gave written informed consent. The study was conducted in accordance with the Helsinki declaration.

Haematological parameters, iron status, and other laboratory measurements in peripheral blood

Laboratory measurements were performed in the laboratories of participating centres. Haematological measurements were made in fresh venous blood with ethylenediaminetetraacetic acid (EDTA). After centrifuging, the material was collected and frozen at −70°C until further laboratory analyses. The definition of anaemia, particular RCI evaluated in this study, and cut-offs applied for the diagnosis of abnormal RCI are presented in *Table 1*. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and MCH concentration (MCHC) were not measured in the Dutch cohorts, and red cell distribution width (RDW) and reticulocyte haemoglobin content (CHR) were not measured in the Dutch cohorts and in one Polish cohort (*Tables 1* and *2*). Both CHR and RDW were measured in a limited number of subjects (*Table 2*). The following blood biomarkers reflecting systemic iron status were measured directly: ferritin (μg/L), serum iron (μg/dL), total iron binding capacity (TIBC, μg/dL), and transferrin (mg/dL). Serum ferritin was measured using an immunoassay based on electrochemiluminescence method. Serum iron and TIBC were assessed using a substrate method. Transferrin measurements were available for most patients and transferrin saturation (TSAT) was reported as a ratio of 0.7217 × serum iron and transferrin, multiplied by 100.[21] When transferrin was not available, TSAT was calculated as a ratio of serum iron and TIBC, multiplied by 100. Iron deficiency was defined as a ferritin level <100 μg/L or serum ferritin 100–299 μg/L in combination with a TSAT <20%, absolute ID as ferritin <100 μg/L, and functional ID as ferritin 100–299 μg/L with transferrin saturation<20%.[9,10] Plasma level of *N*-terminal pro-B-type natriuretic peptide (NT-proBNP, pg/mL) was measured using an immunoassay based on electrochemiluminescence with the Elecsys System (Roche Diagnostics GmbH, Mannheim, Germany). Renal function was assessed estimating creatinine clearance (CrCl, mL/min) using the Cockcroft–Gault equation.[22]

Clinical follow-up

Information regarding survival was obtained directly from patients or their relatives, from the HF clinic databases or from the hospital systems, and was available for 1519 patients (83%). The all-cause death was considered as the primary endpoint in the survival analyses. For the survival analyses the length of follow-up of survivors and patients who died later than 6 years after the enrolment was censored at 2190 days.

Statistical analyses

Most continuous variables had a normal distribution, and were expressed as a mean ± standard deviation (SD) of the mean. The inter-group differences were tested using the analysis of variance (ANOVA) with *post hoc* Scheffé's tests. NT-proBNP, ferritin, and TSAT had a skewed distribution, and were log-transformed (a natural logarithm, ln) before inclusion in further analyses. These variables were expressed as a median with an interquartile range, and the inter-group differences were evaluated after the ln transformation. Categorized variables were expressed as a number and a proportion (%), and the inter-group differences were tested using the chi-square test.

To establish the associations between haemoglobin concentration, RCI (MCV, MCH, MCHC, CHR, and RDW) and iron status (the presence of ID, serum iron, serum ferritin, and TSAT) we constructed a set of multivariable linear regression models (haematological parameter as dependent variable in the model) in which the power of these associations was adjusted for the following clinical variables: (i) age, gender, body mass index (BMI); (ii) the severity [New York Heart Association (NYHA) functional class, left ventricular ejection fraction (LVEF), NT-proBNP] and aetiology of HF (ischaemic vs. non-ischaemic); (iii) renal function (as assessed using CrCl); and (iv) major comorbidities (arterial hypertension and diabetes).

The impact of ID (also absolute and functional ID separately), serum ferritin, and TSAT on long-term mortality was tested: (i) unadjusted (univariable models), and (ii) after the adjustment for key clinical HF prognosticators (predefined: NT-proBNP, NYHA class and CrCl) and either haemoglobin or one of RCI (MCV, MCH, MCHC, CHR, and RDW) (five-variable models). The associations between variables analysed and long-term mortality were evaluated using multivariable Cox proportional hazard regression models.

A *P*-value of <0.05 was considered statistically significant. Statistical analyses were performed using the STATISTICA 12 data analysis software system (StatSoft Inc, Tulsa, OK, USA).

Results

Baseline characteristics of examined patients with heart failure: prevalence of iron deficiency in patients with anaemia or abnormal red cell indices

Clinical characteristics, iron status, and haematological parameters of 1821 examined patients with HF are presented in *Table 2*. Absolute and functional subtypes of ID were detected in 33% and 19% of patients, respectively. Iron deficiency (either absolute or functional) without anaemia, anaemia without ID, and the combination of both [iron deficiency anaemia (IDA)] were diagnosed in 32%, 12%, and 20% of subjects, respectively. In comparison with non-anaemic patients without ID, subjects with IDA were older, had more severe HF symptoms, higher LVEF and plasma NT-proBNP, and lower CrCl (*Table 2*). MCV, MCH, MCHC, and CHR below the lower limit of normal were detected in 4%, 9%, 29%, and 10% of patients, respectively, whereas RDW above the upper limit of

Table 1 Definition of anaemia and abnormal red cell indices in patients with heart failure

Parameter	Abbreviation	Unit	Abnormalities	Reference number
Haemoglobin	Hb	g/dL	Anaemia: <12 g/dL for women and <13 g/dL for men	33
Mean corpuscular volume	MCV	fL	Low MCV: <81 fL for women and <80 fL for men	35, 36
Mean corpuscular haemoglobin	MCH	pg	Low MCH: <26 pg for women and <27 pg for men	35, 36
Mean corpuscular haemoglobin concentration	MCHC	g/dL	Low MCHC: <32 g/dL	35, 36
Mean reticulocyte haemoglobin content	CHR	pg	Low CHR: <28 pg	34, 37, 38
Red cell distribution width	RDW	%	High RDW: >14.5%	39

normal was present in 49% of subjects. Iron deficiency was common comorbidity in HF patients irrespective of either the presence of anaemia or low RCI (*Figures 1* and *2*), from 75% in anaemic subjects with low MCV, MCH, and MCHC, to 36% in patients without anaemia and with these three RCI above the lower limit of normal (*Figure 2*).

Associations between iron status and haemoglobin concentration and red cell indices

In univariable linear regression analyses the presence of ID and lower iron parameters (serum iron, serum ferritin, and TSAT) correlated with lower haemoglobin concentration, MCV, MCH, MCHC, and CHR, and with higher RDW (all $P < 0.001$, *Table 3*).

After the adjustment for relevant clinical variables (age, gender, BMI, severity and aetiology of HF, renal function, and comorbidities), in multivariable linear regression models ID and lower iron parameters remained independently associated with lower haemoglobin, MCV, MCH, MCHC, and CHR, and with higher RDW (all $P < 0.001$, *Table 3*).

Iron status, haematological parameters, and survival in patients with heart failure

Patients without available follow-up (compared with subjects with available follow-up) were more often female (42 vs. 26%, respectively, $P < 0.001$), had less severe HF symptoms (NYHA class I or II: 66% vs. 46%, $P < 0.001$), were older (73 ± 11 years vs. 64 ± 13 years, $P < 0.001$), had higher BMI (28.4 ± 6.1 kg/m^2 vs. 27.5 ± 4.8 kg/m^2, $P = 0.007$), LVEF (46 ± 16% vs. 33 ± 13%, $P < 0.001$), and CrCl (103 ± 77 vs. 91 ± 55, $P < 0.001$), and lower haemoglobin (12.5 ± 1.8 vs. 13.6 ± 1.8 g/dL, $P < 0.001$), and were more often iron-deficient (60% vs. 50%, $P = 0.002$). Importantly, these two groups of patients had similar NT-proBNP (median 1837 vs. 1395 pg/mL, $P = 0.09$).

In 1519 patients studied the mean duration of follow-up (after censoring) was 855 ± 571 days (median: 699 days) whereas the mean time to death (after censoring, $n = 422$) was 604 ± 501 days (median: 480 days). Higher NYHA class and NT-proBNP, and lower CrCl were independent (of each other) predictors of increased all-cause mortality in patients studied [for the three-variable Cox proportional hazard regression model ($\chi^2 = 213.4$, $P < 0.001$): NYHA class hazard ratio (HR) per 1 class increase 1.51, 95% confidence interval (CI) 1.29–1.77, $P < 0.001$; NT-proBNP HR = 1.48 per 1 ln pg/mL increase, 95% CI 1.36–1.62, $P < 0.001$; CrCl HR = 0.95 per 10 mL/min increase, 95% CI 0.93–0.98, $P = 0.002$). The MCV, MCH, MCHC, CHR, and RDW were measured in 1136, 1114, 1114, 276, and 605 patients with available follow-up, respectively. In univariable Cox proportional hazard regression analyses the following haematological parameters were associated with increased all-cause mortality: lower haemoglobin concentration (HR = 0.86 per 1 g/dL increase, 95% CI 0.81–0.91, $P < 0.001$), MCH (HR = 0.94 per 1 pg increase, 95% CI 0.90–0.98, $P = 0.008$), MCHC (HR = 0.89 per 1 g/dL increase, 95% CI 0.83–0.95, $P < 0.001$), CHR (HR = 0.91 per 1 pg increase, 95% CI 0.86–0.97, $P = 0.002$), and higher RDW (HR = 1.16 per 1% increase, 95% CI 1.10–1.24, $P < 0.001$).

In univariable Cox proportional hazard regression analyses the presence of ID (also absolute and functional ID separately) and lower TSAT (but not serum ferritin) predicted higher long-term all-cause mortality in patients with HF (*Table 4*, all $P < 0.05$). Importantly, when adjusted for aforementioned relevant HF prognosticators (NT-proBNP, NYHA class, CrCl) and either MCH or MCHC (but not haemoglobin concentration, CHR, or RDW) there was still a trend towards higher mortality in patients with either ID or lower TSAT (all $P < 0.1$) (*Table 4*).

Discussion

The major findings of this study are: (i) despite evident associations between iron status and RCI, ID is a common comorbid condition in HF patients irrespective of the presence of anaemia and/or abnormal RCI; (ii) ID predicts increased long-term mortality in these patients, which is partially independent of low RCI.

Owing to the traditional view of ID as a nutritional deficiency leading to IDA, low RCI have been considered sensitive indicators of decreased iron availability for haematopoietic tissues for more than 50 years.[23] Indeed, low MCV, MCH, and MCHC reflect the advanced stage of iron-restricted erythropoiesis within the bone marrow, and the picture of microcytic and hypochromic anaemia constitutes the typical laboratory presentation of IDA.[14,15,17]

Table 2 Baseline characteristics of patients with heart failure according to the presence of anaemia and/or iron deficiency

Variables	*n*	All patients with HF	No ID and no anaemia	ID only	Anaemia only	ID and anaemia
Age, years	1821	66 ± 13	61 ± 13	66 ± 13***	69 ± 12***	72 ± 11***
Sex, male	1821	1298 (71)	533 (81)	381 (66)***	155 (72)**	229 (62)***
BMI, kg/m^2	1772	27.6 ± 5.1	28.0 ± 5.0	27.5 ± 4.9	26.8 ± 4.7	27.8 ± 5.6
SBP, mmHg	1736	121 ± 21	119 ± 20	121 ± 22	120 ± 24	123 ± 21*
Heart rate, b.p.m.	1740	76 ± 15	77 ± 15	76 ± 15	76 ± 14	75 ± 14
NYHA class, I,II,III,IV	1821	195/706/802/118 (11/39/44/6)	88/307/237/27 (13/47/36/4)	55/211/278/31 (10/37/48/5)***	24/81/95/16 (11/38/44/7)*	28/107/192/44 (8/29/52/12)***
HF aetiology, CAD	1821	1042 (57)	376 (57)	332 (58)	124 (57)	210 (57)
LVEF, %	1821	35 ± 15	32 ± 13	34 ± 14	38 ± 17***	41 ± 17***
NT-proBNP, pg/mL	1804	1440 (575–3553)	1098 (461–2360)	1409 (506–3481)*	1898 (794–5746)***	2185 (977–4966)***
CrCl, mL/min	1800	93 ± 59	102 ± 62	91 ± 46**	83 ± 52***	84 ± 72***
Arterial hypertension, yes	1813	1065 (59)	349 (53)	310 (54)	149 (69)***	257 (70)***
Diabetes, yes	1805	664 (37)	197 (30)	184 (32)	92 (43)***	191 (52)***
Treatment						
ACEI or/and ARB, yes	1802	1599 (89)	618 (94)	518 (91)*	177 (84)***	286 (78)***
β-blocker, yes	1816	1642 (90)	608 (92)	516 (90)	197 (91)	321 (87)**
Aldosterone antagonist, yes	1612	821 (51)	330 (55)	238 (50)	102 (51)	151 (46)**
Digoxin, yes	1718	420 (24)	194 (31)	124 (24)*	46 (22)*	56 (15)***
Statin, yes	1809	1164 (64)	433 (66)	364 (63)	139 (65)	228 (62)
Loop diuretic, yes	1820	1455 (80)	486 (74)	460 (80)**	181 (84)**	328 (88)***
Antiplatelets, yes	1817	927 (51)	340 (52)	287 (50)	115 (53)	185 (50)
Anticoagulants, yes	1719	733 (43)	253 (40)	225 (44)	91 (43)	164 (45)
Indices of iron status						
Iron, μg/dL	1821	79 ± 44	107 ± 43	66 ± 36***	78 ± 45***	47 ± 23***
Ferritin, μg/L	1821	150 (79–274)	256 (164–397)	83 (54–128)***	319 (198–472)*	78 (46–142)***
TSAT, %	1821	21 (14–31)	30 (24–40)	17 (13–23)***	24 (20–30)***	13 (9–17)***
Haemoglobin and RCI						
Haemoglobin, g/dL	1821	13.4 ± 1.9	14.6 ± 1.3	14.1 ± 1.1***	11.4 ± 1.3***	11.3 ± 1.1***
MCV, fL	1437	91.0 ± 6.0	92.0 ± 5.1	91.0 ± 5.6	91.9 ± 7.2	88.7 ± 6.2***
MCH, pg	1407	29.9 ± 2.6	31.0 ± 2.1	29.9 ± 2.2***	29.9 ± 3.1***	28.2 ± 2.5***
MCHC, g/dL	1407	32.9 ± 1.9	33.8 ± 1.8	32.9 ± 1.5***	32.5 ± 1.9***	31.8 ± 1.6***
CHR, pg	563	32.3 ± 3.6	33.5 ± 2.7	32.7 ± 2.5	32.7 ± 4.3	30.5 ± 4.2***
RDW, %	897	15 ± 2	14 ± 2	15 ± 2**	15 ± 2***	16 ± 2***

Data are presented as a mean ± standard deviation of the mean, a median (with lower and upper quartiles) or n (with %), where appropriate. n, number of patients for whom data were available. Anaemia was defined as haemoglobin <12 g/dL in women and <13 g/dL in men. ID was defined as ferritin <100 mg/L, or 100–299 mg/L with transferrin saturation <20%.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; CHR, mean reticulocyte haemoglobin content; CrCl, creatinine clearance; HF, heart failure; ID, iron deficiency; LVEF, left ventricular ejection fraction; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; NT-proBNP, *N*-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; RCI, red cell indices; RDW, red cell distribution width; SBP, systolic blood pressure; TSAT, transferrin saturation.

P-values for the comparison with the group 'No ID and no anaemia': $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (*post hoc*).

For details, see the Methods section.

Increased RDW is another indicator of IDA, but this parameter is also elevated in megaloblastic anaemia.[3,24] In comparison with aforementioned parameters, decreased haemoglobin in reticulocytes, as reflected by low CHR, characterizes the very early stages of defective erythropoiesis due to ID, and is of particular clinical importance as it allows one to monitor the response to parenteral iron therapy.[15] Importantly, in the present study, comprising a large international cohort of HF patients with both reduced and preserved LVEF, we have shown that although haemoglobin concentration and particular RCI (MCV, MCH, MCHC, CHR, and RDW) closely correlate with different iron parameters (the presence of ID, serum iron, serum ferritin, and TSAT) independently of other clinical and laboratory variables (including aetiology and severity of HF and important comorbidities), ID

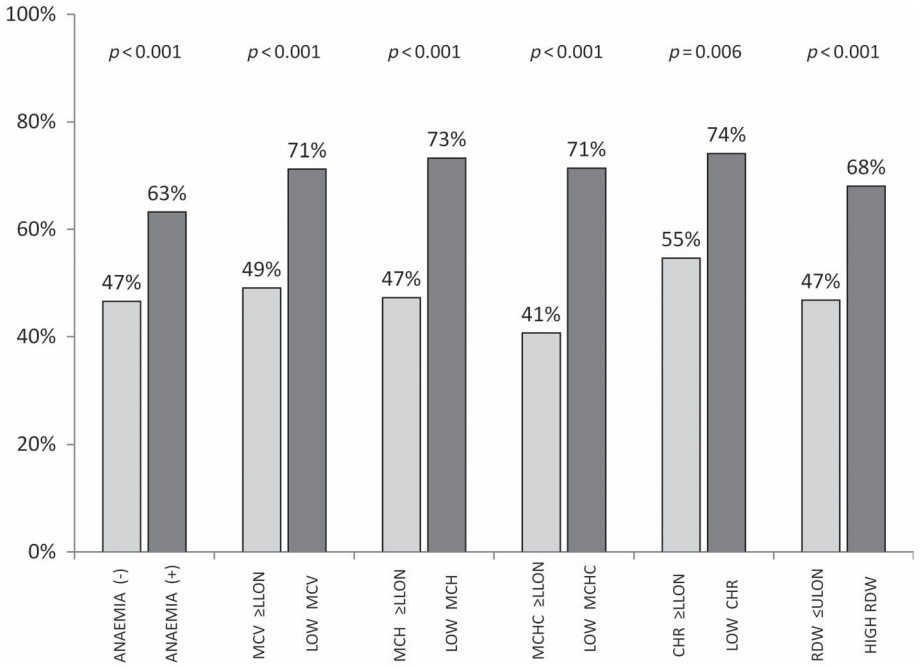

Figure 1 The prevalence of iron deficiency in patients with heart failure according to the presence of anaemia or abnormal red cell indices. LLON, lower limit of normal; ULON, upper limit of normal; MCH, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; CHR, mean reticulocyte haemoglobin content; RDW, red cell distribution width. (For the definition of iron deficiency, anaemia, and abnormal red cell indices, see the Methods section and *Table 1*).

also constitutes a frequent comorbidity in patients without any haematological abnormalities. It needs to be emphasized that even in non-anaemic [according to the World Health Organization (WHO) definition] patients with MCV, MCH, and MCHC above the lower limit of normal, the prevalence of ID reached 36%. An observed considerable prevalence of ID irrespective of the presence of anaemia or abnormal RCI suggests that although disordered iron homeostasis represents one of the causes of anaemia in HF,[2,25] many patients with cardiac failure and concomitant ID will not develop haematological abnormalities. Indeed, in recent years complex derangements regarding iron status in HF have been elucidated,[1,3] and the traditional view of ID as a leading cause of anaemia[25] in these patients has been revised.[2] With regard to pathophysiology, ID contributes to the cardiorenal–anaemia axis in patients with HF,[26] and the complex interplay between cardiac failure, renal dysfunction, ID, and anaemia has been emphasized in a paper by Macdougall *et al.*,[27] in which the authors introduced the term cardiorenal–anaemia–iron deficiency syndrome (CRAIDS). Nevertheless, the aetiology of anaemia in HF is multifactorial,[2] with several contributing and overlapping pathomechanisms such as renal impairment, systemic inflammation, ID, and haemodilution, to name but a few.[2,28,29] Importantly, the present study confirms that in patients with HF ID should not only be perceived as a cause of anaemia, but an equivalent comorbidity that can occur without haematological abnormalities, and is generally more frequent than anaemia.[3,5,30]

In the present study we have also demonstrated that in patients with HF with either reduced or preserved LVEF, concomitant ID predicts increased long-term all-cause mortality, which is partially independent of RCI. In univariable Cox proportional hazard regression analyses both iron status (ID and lower TSAT, but not serum ferritin) and RCI (lower haemoglobin, MCH, MCHC, CHR, and higher RDW, but not MCV) predicted increased long-term mortality. Further, in multivariable analyses there was still a trend towards higher mortality in patients with either ID (including functional but not absolute subtype of this comorbidity separately) or lower TSAT (all $P < 0.1$), when adjusted for relevant clinical HF prognosticators (neurohormonal activation, severity of HF symptoms and renal function) and MCH or MCHC (but not haemoglobin, CHR or RDW). Our results are consistent with previous findings demonstrating the detrimental prognostic consequences of ID in patients with either stable or acute HF.[3,6,8,31] Importantly, in the patients with HF studied the trend towards higher mortality was associated more with functional than absolute ID, and serum ferritin, reflecting body iron stores, was not related to survival in this population. From the pathophysiological point of view, functional ID results from reduced availability of iron for iron-utilizing cells (e.g. erythropoietic), and is promoted by increased systemic inflammation.[2] Importantly, although patients with more severe HF symptoms are characterized by lower ferritin, TSAT, hepcidin (key regulator of iron metabolism), and haemoglobin, and by increased circulating inflammatory biomarkers, the associations between iron and inflammatory parameters

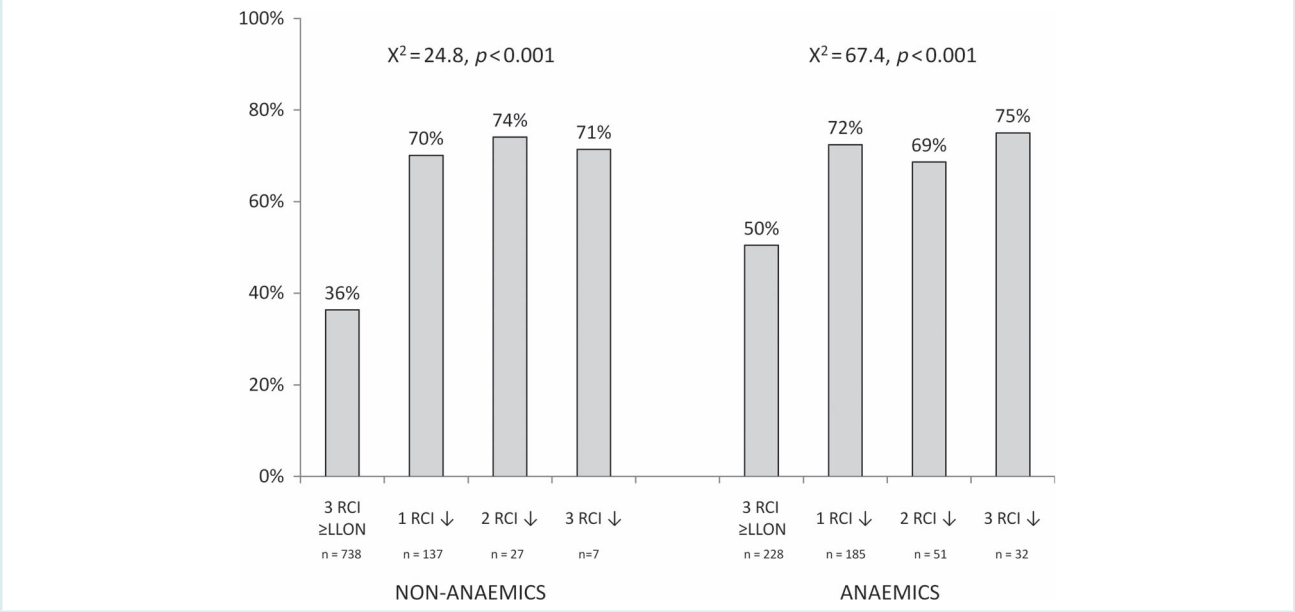

Figure 2 The prevalence of iron deficiency in patients with heart failure according to the presence of anaemia and the number of decreased red cell indices (RCI) (of the following three: mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration). LLON, lower limit of normal. For the definition of iron deficiency, anaemia, and decreased RCI, see the Methods section and *Table 1*.

Table 3 Associations between iron status and haemoglobin concentration and red cell indices in patients with heart failure

		Haemoglobin and red cell indices (dependent variable)					
ID and iron parameters	**Applied adjustment†**	**Haemoglobin (g/dL)**	**MCV (fL)**	**MCH (pg)**	**MCHC (g/dL)**	**CHR (pg)**	**RDW (%)**
ID (yes vs. no)	None	−0.21***	−0.17***	−0.31***	−0.28***	−0.24***	0.21***
ID (yes vs. no)	10 clinical variables	−0.10***	−0.17***	−0.25***	−0.19***	−0.23***	0.14***
Iron (μg/dL)	None	0.39***	0.19***	0.38***	0.36***	0.34***	−0.28***
Iron (μg/dL)	10 clinical variables	0.26***	0.21***	0.30***	0.23***	0.36***	−0.20***
Ferritin (ln μg/L)	None	0.16***	0.15***	0.25***	0.21***	0.21***	−0.14***
Ferritin (ln μg/L)	10 clinical variables	0.08***	0.16***	0.22***	0.16***	0.22***	−0.12***
TSAT (ln %)	None	0.35***	0.29***	0.46***	0.38***	0.48***	−0.33***
TSAT (ln %)	10 clinical variables	0.22***	0.32***	0.42***	0.27***	0.50***	−0.24***

Data are presented as standardized regression coefficients β (both in univariable and multivariable models) between haematological parameters (dependent variable in the multivariable model) and indices of iron status.
CHR, mean reticulocyte haemoglobin content; ID, iron deficiency; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RDW, red cell distribution width; TSAT, transferrin saturation.
†10 clinical variables: age, gender, body mass index, New York Heart Association functional class, aetiology of heart failure, left ventricular ejection fraction, *N*-terminal pro-B-type natriuretic peptide [natural logarithm (ln)], estimated creatinine clearance, concomitant arterial hypertension, and diabetes. Anaemia was defined as haemoglobin <12 g/dL in women and <13 g/dL in men. ID was defined as ferritin <100 μg/L, or 100–299 μg/L with TSAT <20%.
***$P < 0.001$. For details see the Methods section.

are more complex than would be expected: for example, serum high-sensitivity C-reactive protein correlates neither with ferritin nor with hepcidin in these patients.[31] The present study confirms, that comorbid ID is not simply the feature of advanced HF, and detrimental impact of ID on long-term survival in these patients is partially independent of either HF severity or decreased RCI. Further studies regarding the impact of ID on the natural history of HF are warranted.

It should be acknowledged that although current 2016 European Society of Cardiology guidelines for the diagnosis and treatment of acute and chronic HF[32] clearly recommend searching for comorbidities such as ID in all patients with newly diagnosed HF (class of recommendation I, level of evidence C), in daily clinical practice, patients with normal RCI and without anaemia have rarely assessed ferritin and TSAT. The results of the present study confirm that all patients with HF should be routinely screened

Table 4 Iron status and long-term all-cause mortality in patients with heart failure (univariable and multivariable Cox proportional hazard regression models)

	Iron status																			
	ID (yes vs. no)				Absolute ID (yes vs. no)***				Functional ID (yes vs. no)****				Ferritin (1 ln µg/L)				TSAT (1 ln %)			
Adjustment	HR	95% CI	WS	*P*-value	HR	95% CI	WS	*P*-value	HR	95% CI	WS	*P*-value	HR	95% CI	WS	P-value	HR	95% CI	WS	P-value
None (univariable analysis)	1.34	1.10–1.62	8.72	0.0032	1.26	1.01–1.56	4.37	0.0367	1.50	1.16–1.93	9.87	0.0017	1.01	0.91–1.12	0.03	0.8710	0.75	0.67–0.84	23.7	<0.0001
Three clinical variables* + haemoglobin	1.12	0.92–1.37	1.31	0.2522	1.06	0.84–1.32	0.23	0.6323	1.25	0.97–1.62	2.91	0.0883	-	-	-	-	0.98	0.87–1.10	0.14	0.7088
Three clinical variables* + MCH**	1.26	0.98–1.61	3.28	0.0703	1.23	0.93–1.62	2.05	0.1518	1.40	0.99–1.96	3.72	0.0537	-	-	-	-	0.80	0.64–1.01	3.65	0.0561
Three clinical variables* + MCHC**	1.23	0.97–1.58	2.82	0.0934	1.21	0.92–1.60	1.85	0.1734	1.34	0.95–1.88	2.82	0.0933	-	-	-	-	0.82	0.66–1.02	3.02	0.0821
Three clinical variables* + CHR**	1.25	0.80–1.96	0.93	0.3337	1.30	0.78–2.18	1.03	0.3112	1.29	0.73–2.27	0.77	0.3803	-	-	-	-	0.77	0.49–1.21	1.29	0.2558
Three clinical variables* + RDW**	0.90	0.63–1.27	0.37	0.5443	0.88	0.60–1.31	0.38	0.5383	0.91	0.58–1.45	0.14	0.7046	-	-	-	-	0.97	0.71–1.34	0.03	0.8678

CHR, mean reticulocyte haemoglobin content; CI, confidence interval; HR, hazard ratio; ID, iron deficiency; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RDW, red cell distribution width; WS, Wald's statistics.

*Three clinical variables: *N*-terminal pro-B-type natriuretic peptide (unit: 1 ln pg/mL), New York Heart Association class (1 class), and estimated creatinine clearance (1 mL/min).

**MCV, MCH, MCHC, CHR and RDW were not measured in all patients with available follow-up (see the Results section). ID was defined as ferritin <100 µg/L. or 100–299 µg/L with transferrin saturation <20%; absolute ID as ferritin <100 µg/L; and functional ID as ferritin 100–299 µg/L with transferrin saturation <20%. For details see the Methods section.

***Patients with functional ID excluded from the analyses. ****Patients with absolute ID excluded from the analyses.

for ID, irrespective of the presence or absence of haematological abnormalities, as these pathologies are not simply parallel. Moreover, we have shown that a detrimental impact of ID on long-term prognosis in HF patients is partially independent of haematological abnormalities (i.e. decreased RCI). This is another premise for the routine assessment of iron parameters in HF patients to improve the risk stratification process in subjects who are usually not suspected of ID. An active screening for ID in patients with symptomatic HF with reduced LVEF would allow clinicians to identify more potential beneficiaries of intravenous iron therapy.[32] Importantly, there is no direct evidence of whether intravenous iron therapy improves outcomes in HF patients (also irrespective of baseline haematological status).

Limitations

It should be emphasized that there is no universal lower limit of normal blood haemoglobin concentration (i.e. the definition of anaemia). In the present study the definition of anaemia was based on the WHO report (haemoglobin concentration <12 g/dL for women and <13 g/dL for men) from 1968,[33] and the same definition is mentioned in the 2016 ESC guidelines for the diagnosis and treatment of acute and chronic HF.[32] Although this definition of anaemia is widely accepted and commonly applied in epidemiological studies (including studies regarding HF patients), the appropriateness of these cut-offs remains very controversial and has been criticized.[34] Analogously to haemoglobin, in the present study, the lower limits of normal MCV, MCH, and MCHC were defined according to the WHO document based on US Second National Health and Nutrition Examination Survey (NHANES II)[34,36] (*Table 1*), and these cut-offs also may not be optimal for the contemporary Europeans with HF.

Furthermore, the follow-up was available for 1519 patients only (83%), which is a potential source of bias. Compared with subjects included in survival analyses, patients without available follow-up had a different clinical profile: they were older and more often female, had less severe HF symptoms, higher LVEF, better renal function, lower haemoglobin, and, finally, they were more often iron-deficient. Importantly, MCV, MCH, MCHC, CHR, and RDW were not measured in all patients with available follow-up (see the Results section).

Another limitation of the study is that although patients did not receive blood transfusions, erythropoietin therapy, or intravenous iron therapy at the time of inclusion, we do not have data regarding potential iron therapy (either intravenous or oral) or therapy with erythropoiesis-stimulating agents during the follow-up period.

Conclusions

The present study confirms that, in patients with HF, ID should not only be perceived as a cause of anaemia, but an equivalent comorbid condition that can occur without haematological abnormalities regarding RCI or haemoglobin concentration. Importantly, the detrimental impact of ID on long-term survival in HF patients is partially independent of decreased RCI. Patients with HF should be routinely screened for ID irrespective of the presence of anaemia or abnormal RCI.

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