Parámetros hematológicos descuidados en el pronóstico de la insuficiencia cardíaca-Evidencia del estudio REFERENCE

Neglected hematological parameters in heart failure prognosis – Disclosures from the REFERENCE study

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ABSTRACT

Aims: In heart failure patients, anemia and iron deficiency are predictors of poor outcome. We studied the association of anemia, iron deficiency and related hematological parameters with short-term rehospitalization, short-term all-cause mortality and end of follow-up all-cause mortality in heart failure patients.

Material and Methods: Anemia, iron deficiency, red cell distribution width and erythropoietin were assessed in patients hospitalized with acute decompensated heart failure.

Univariate Cox proportional hazard model was used to assess the relationship between variables and outcomes.

Results: 65 patients were followed for a median of 13.7 (Q1-Q3 6.7-18.9) months. Mean age was 79.2 (SD 10.8) years. The mean left ventricular ejection fraction was 50.38 ± 19.07 %. Variables associated with an increased risk for short-term rehospitalization were red cell distribution width (HR 1.35; 95% CI 1.16-1.58), anemia (HR 3.81; 95% CI 1.29-11.28) and anemia with iron deficiency (HR 3.50; 95% CI 1.30-9.38). Increased risk for short-term mortality was associated with red cell distribution width (HR 1.83; 95% CI 1.29-2.59), erythropoietin (HR 1.38; 95% CI 1.04-1.82), absolute iron deficiency (HR 7.22; 95% CI 1.50-34.81) and anemia with iron deficiency (HR 4.48; 95% CI 1.26-15.88). Variables associated with increased risk for end of follow-up mortality were red cell distribution width (HR 1.31; 95% CI 1.12-1.54) and erythropoietin (HR 1.29; 95% CI 1.11-1.49).

Conclusions: Conclusions: Anemia and red cell distribution width correlated with higher risk for short-term rehospitalization. Absolute iron deficiency, red cell distribution width and erythropoietin were associated with higher risk for short-term mortality. Red cell distribution width and erythropoietin were associated with higher risk for end of follow-up mortality.

Keywords: Acute Heart Failure; Prognosis; Anemia; Iron Deficiency; Red Cell Distribution Width; Erythropoietin.

INTRODUCTION

Heart failure (HF) is the leading cause of hospital admissions in Europe and in the United States of America, accounting for more than 1 million hospitalizations annually[1].

Despite management consentaneous with the recommended guidelines, diagnostic advances, innovative drugs and new therapeutic devices, short-term prognosis has failed to improve as approximately one in four HF patients are readmitted within the first 30 days[2] and up to 30% are rehospitalized 60 to 90 days post-discharge [3].

Furthermore, the mortality rate 60 to 90 days post-discharge is around 15% [4].

Anemia and iron deficiency (ID) are common comorbidities that often coincide in patients with HF[5,6].

Both conditions have been shown to be associated with an increased risk for rehospitalization and mortality among patients with HF[6, 7, 8].

Moreover, hematological parameters related with anemia and ID, such as Red Cell Distribution Width (RDW)[9] and erythropoietin (EPO)[10, 11], have been linked to increased mortality in patients with HF.

We believe that the recognition and treatment of comorbidities that influence the outcome of HF, beyond HF-specific therapy, may further contribute to ameliorate HF prognosis.

Hence, we performed a prospective cohort study named PREdictors oF Early REadmission iN Chronic HEart Failure (REFERENCE) to investigate whether anemia, ID and related hematological parameters are associated with short-term rehospitalization, short-term mortality and end of follow-up mortality in HF patients.

MATERIAL AND METHODS

Study design and population

For this prospective observational cohort study patients were recruited consecutively for a period of 12 months from an Internal Medicine ward.

Inclusion criteria were age ≥18 years old and hospitalization due to acute decompensated heart failure (ADHF) in class III or IV of New York Heart Association.

Patients were excluded if they had chronic kidney disease with glomerular filtration rate < 30 ml/min/1.73 m2 (calculated with the Modification of Diet in Renal Disease score) or were under renal replacement therapy, moderate or severe hepatic impairment (calculated with the Child-Pugh score), in-hospital death in the first hospitalization, hospital discharge against medical advice or active neoplasm with or without metastasis.

All patients gave written informed consent.

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Cómo citar este artículo: Barbosa M, Matos A, Bicho M, Menezes Falcão L
Neglected hematological parameters in heart failure prognosis – Disclosures from the REFERENCE study. Galicia Clin 2022; 83-2: 20-31
Recibido: 05/12/2020 ; Aceptado: 28/04/2022 // https://doi.org/10.22546/65/2413

The study was approved by an Institutional Review Board (Academic Medical Center Ethics Committee) and was conducted in accordance with the Declaration of Helsinki and the Oviedo Convention.

Laboratory measurements and definitions

The diagnosis of HF was based on the European Society of Cardiology (ESC) guidelines[12].

Erythropoietin was quantified in plasma using pre-coated human ELISA kits (R&D Systems, Abingdon, USA). The reference range for EPO was 2.5-200 mIU/mL and the minimum detectable amount was 0.6 mIU/mL.

Anemia was defined according to the World Health Organization[13].

Absolute and functional ID associated with chronic heart failure (CHF) were defined according to the ESC[12].

Subgroup analysis was performed according to the left ventricular ejection fraction (LVEF) in light of the current ESC guidelines[12].

Treatment, including intravenous ferric carboxymaltose, was optimized following the ESC guidelines[12].

None of the patients required red blood cells transfusion.

Outcomes

Outcomes for this study were short-term rehospitalization, short-term all-cause mortality and end of follow-up all-cause mortality. Short-term rehospitalization was defined as rehospitalization within 90 days of hospital discharge. Short-term mortality was defined as death that occurred within 90 days after hospital discharge. End of follow-up mortality was defined as death that occurred during the whole study period.

Statistical analysis

Convenience sampling was used and no sample size calculation was performed. Categorical variables were summarized by relative and absolute frequencies and compared using the chi-squared test or Fisher's Exact test.

Continuous variables were summarized by mean, standard deviation, median, first and third quartiles (Q1-Q3). Shapiro-Wilk test was used to assess the normality of continuous variables. Comparisons between patients with or without an event of interest were performed using the t-test or Wilcoxon Rank test, as applicable.

Short-term rehospitalization, short-term mortality and end of follow-up mortality were considered as stratification variables.

A survival analysis was performed for the events of interest.

For each endpoint, Kaplan-Meier survival estimates were calculated and plotted for each categorical variable. Log-rank tests were used to compare survival probabilities in each of the considered variables. A univariate Cox proportional hazards model was fitted to the data to obtain HR and 95% CI for each variable.

The proportional hazards assumption was tested using Schoenfeld residuals. All analyses were conducted at an overall significance level of 5%. No imputation was performed on missing data. No adjustments for multiplicity were performed.

RESULTS

In total, 70 patients were admitted due to ADHF. During follow-up 5 patients were diagnosed with active cancer and were excluded, leaving 65 patients who were followed up for a median (Q1-Q3) of 13.7 (6.7-18.9) months. The mean (SD) age of the study population was 79.2 (10.8) years, 56.9% were female and the mean (SD) LVEF was 50.4 (19.1) %.

Among the patients, 58.5% had anemia and 46.2% had iron deficiency.

Baseline characteristics are depicted in Table 1 and descriptive analysis for the study population according to short-term rehospitalization, short-term mortality and end of follow-up mortality is shown in Table 2.

Mean hemoglobin concentration was significantly lower in patients who had a short- term rehospitalization compared to those with no short-term rehospitalizations ($p < 0.05$). Likewise, anemia was more prevalent in patients with a short-term rehospitalization compared to non-rehospitalized patients ($p < 0.01$).

Furthermore, absolute ID was more than twice as common in the patients with short- term mortality compared to those who survived the first 90 days ($p < 0.01$, Table 2).

Anemia with ID was more prevalent among patients who had a short-term rehospitalization compared to the patients who were not rehospitalized ($p < 0.05$, Table 1). Anemia with ID was also more common among patients who died within the first 90 days compared to those who survived during this period ($p < 0.05$, Table 2). In addition, anemia with absolute ID was more common among patients with short-term mortality compared to patients who survived ($p < 0.01$, Table 2).

Moreover, patients with a short-term rehospitalization had higher median RDW compared to patients who did not have a short-term readmission ($p < 0.05$, Table 2). Higher median RDW was also observed in patients with short-term mortality compared to patients who survived the first 90 days post-discharge ($p < 0.01$, Table 2) and in patients who died during the follow-up compared to those that survived ($p < 0.05$, Table 2).

The median level of EPO was more than twice as high in patients who died early compared to patients who remained alive during the first 90 days post-discharge ($p < 0.05$, Table 2). The median concentration of EPO was also higher among the patients who died during the follow-up compared to the patients who survived ($p < 0.001$, Table 2).

Univariate Cox proportional hazards analysis was used to study the relationship between the study outcomes and anemia, ID and related hematological parameters. The results from the analysis are shown in Table 3.

Hemoglobin concentration, per increments of 1 g/dL, was associated with a lowered risk for short-term rehospitalization (HR 0.78; 95% CI 0.64-0.96; $p < 0.05$). We also found that anemia was associated with an increased risk of short-term rehospitalization (HR 3.81; 95% CI 1.29-11.28; $p < 0.05$). Absolute ID was associated with an increased risk for short-term mortality (HR 7.22; 95% CI 1.5-34.81; $p < 0.05$). Anemia with ID was associated with an increased risk of short-term rehospitalization (HR 3.50; 95% CI 1.3-9.38; $p < 0.05$) and short-term mortality (HR 4.48; 95% CI 1.26-15.88; $p < 0.05$). In addition, anemia with absolute ID was associated with an increased risk for short-term rehospitalization (HR 3.14; 95% CI 1.13-8.74; $p < 0.05$) and short-term mortality (HR 6.14; 95% CI 1.73-21.8; $p < 0.01$).

Subgroup analysis showed that in the HFrEF subgroup, each serum iron increase of 10 ug/dL was associated with a reduced

Table 1. Baseline characteristics

Characteristics	Patients (n=65)
Age, mean (SD)	79.2 ± 10.8
Female Gender, n (%)	37 (56.9)
Hypertension, n (%)	58 (89.2)
Admission Systolic Blood Pressure, median	145.0 (121 - 163)
Type 2 Diabetes, n (%)	25 (38.5)
Dyslipidemia, n (%)	41 (63.1)
Obesity, n (%)	17 (26.2)
Atrial Fibrillation, n (%)	28 (43.1)
Ischemic Heart Disease, n (%)	22 (33.8)
Anemia, n (%)	38 (58.5)
Iron deficiency, n (%)	30 (46.2)
Chronic Kidney Disease, n (%)	34 (52.3)
GFR (Baseline), median	57.8 (43.8 - 82.2)
GFR (Admission), median	47.9 (33.2 - 68.1)
LVEF, mean (SD)	50.38 ± 19.07
NYHA class III, n (%)	43 (66.2)
ACE Inhibitor, n (%)	43 (66.2)
Beta Blocker, n (%)	38 (58.5)
Mineralocorticoid Receptor Antagonists n (%)	19 (29.2)
Angiotensin II Receptor Blocker, n (%)	11 (16.9)
Loop Diuretic, n (%)	54 (83.1)
Digoxin, n (%)	8 (12.3)
NT-proBNP (Admission), median	5701.0 (1867 - 11961)
NT-proBNP (Discharge), median	2837.0 (520 - 5085)

Values are median (IQR), n (%) or mean ± SD.
IQR: interquartile range and minimum/maximum, SD: standard deviation, CVD: cardiovascular disease, GFR: glomerular filtration rate, LVEF: left ventricular ejection fraction, NYHA: New York Heart Association, ACE: Angiotensin-Converting-Enzyme.

risk of short-term mortality (HR 0.34; 95% CI: 0.12-0.98; $p = 0.046$) and anemia was associated with an increased risk for end of follow-up mortality (HR 11.14; 95% CI 1.35-92.07; $p = 0.025$) (Supplementary Table 1).

As well, each 10% increment of RDW was associated with an increased risk for short- term rehospitalization (HR 1.35; 95% CI 1.16-1.58; $p < 0.001$), short-term mortality (HR 1.83; 95% CI 1.29-2.59; $p < 0.001$) and end of follow-up mortality (HR 1.31; 95% CI 1.12-1.54; $p < 0.001$) (Table 3).

Each 10 mU/mL increment of EPO concentration was associated with an increased risk of short-term mortality (HR 1.38; 95% CI 1.04-1.82; $p < 0.05$) and end of follow-up mortality (HR 1.29; 95% CI 1.11-1.49; $p < 0.01$) (Table 3). A trend towards increased risk of short-term rehospitalization was associated with elevated EPO concentration in both the total study population (HR: 1.06, 95% CI: 0.99-1.14, $p = 0.074$, Table 3) and in the HFrEF subgroup (HR: 1.08, 95% CI: 0.99-1.19, $p = 0.074$, Supplementary Table 1).

Kaplan-Meier survival curves showed that the rate of short-term rehospitalization was significantly higher for patients with anemia (Log rank = 0.0094), as shown in Figure 1.

Figure 2 shows that survival was significantly worse for patients with ID compared to patients without ID (Log rank = 0.0042).

DISCUSSION

Mild anemia is a common condition in patients suffering from chronic diseases, of which HF is no exception.[5] Moreover, the prevalence of anemia increases with more severe HF.[5]

In patients with CHF, ID is common, either with or without concurrent anemia[5, 6] and is related to disease severity[14].

Among patients with ADHF, the prevalence of ID ranges from 50% to 80%.[15-17]

Figure 1 - Kaplan Meier survival curves for short-term rehospitalization rate according to anemia status

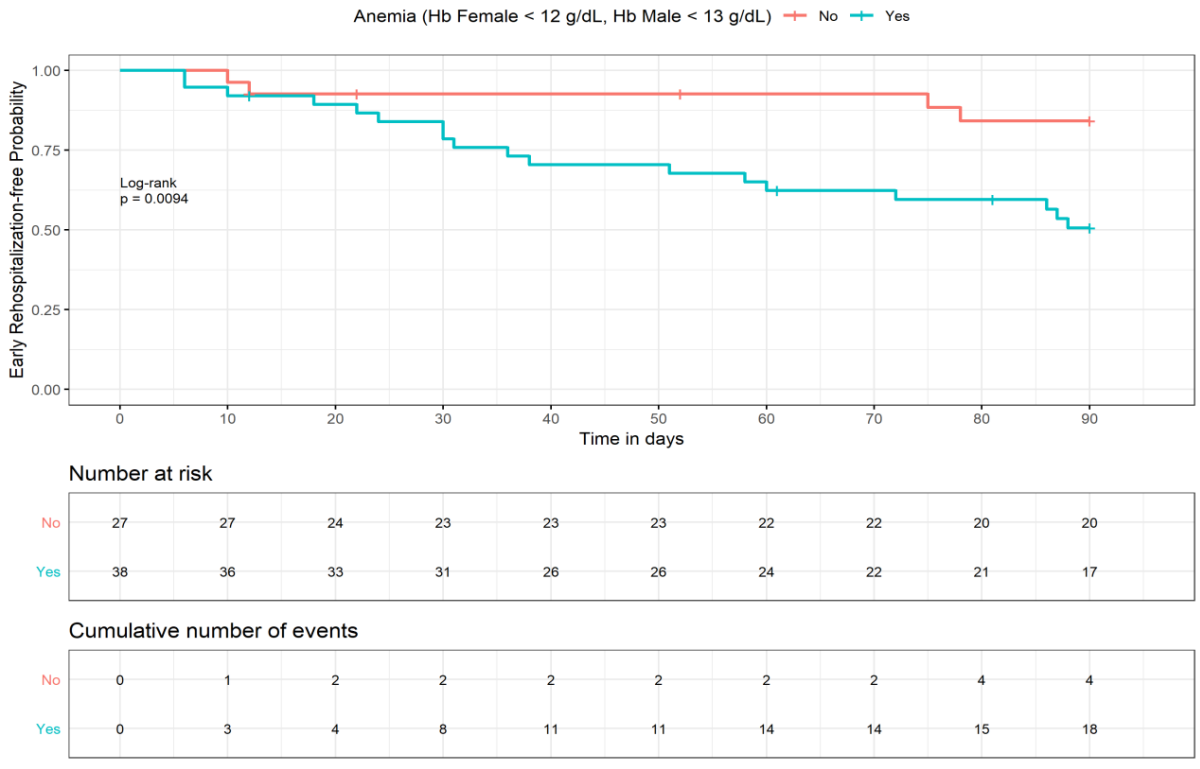


Table 2. Descriptive analysis for the study population

Characteristics	Short-term rehospitalization		Short-term mortality		End of follow-up mortality	
	No (N = 43)	Yes (N = 22)	No (N = 54)	Yes (N = 11)	No (N = 38)	Yes (N = 27)
Age, mean (SD), years	79.8 (10.8) [43]	78.1 (10.9) [22]	78.9 (11.1) [54]	80.7 (9.0) [11]	80.6 (9.4) [38]	77.4 (12.4) [27]
Sex, n (%)						
Female	25 (58.1) [43]	12 (54.5) [22]	30 (55.6) [54]	7 (63.6) [11]	23 (60.5) [38]	14 (51.9) [27]
Male	18 (41.9) [43]	10 (45.5) [22]	24 (44.4) [54]	4 (36.4) [11]	15 (39.5) [38]	13 (48.1) [27]
NYHA, n (%)						
NYHA III	15 (34.9) [43]	8 (36.4) [22]	17 (31.5) [54]	4 (36.4) [11]	10 (26.3) [38]	12 (44.4) [27]
NYHA IV	28 (65.1) [43]	14 (63.6) [22]	37 (68.5) [54]	7 (63.6) [11]	28 (73.7) [38]	15 (55.6) [27]
LVEF, n (%)						
Preserved (> 49%)	24 (55.8) [43]	11 (50) [22]	31 (57.4) [54]	6 (54.5) [11]	23 (60.5) [38]	12 (44.4) [27]
Midrange (40-49%)	9 (20.9) [43]	4 (18.2) [22]	10 (18.5) [54]	2 (18.2) [11]	6 (15.8) [38]	7 (25.9) [27]
Reduced (< 40%)	10 (23.3) [43]	7 (31.8) [22]	13 (24.1) [54]	3 (27.3) [11]	9 (23.7) [38]	8 (29.6) [27]
Comorbidities						
Diabetes mellitus type 2, n (%)	15(34.9) [43]	10 (45.5) [22]	20 (37.0) [54]	5 (45.5) [11]	13 (34.2) [38]	12 (44.4) [27]
Obesity (BMI > 30 kg/m2), n (%)	9 (20.9) [43]	7 (31.8) [21]	14 (25.9) [53]	1 (9.1) [8]	12 (31.6) [38]	5 (18.5) [27]
Arterial hypertension, n (%)	37(86.0) [43]	21 (95.5) [22]	49 (90.7) [54]	9 (81.8) [11]	35 (92.1) [38]	23 (85.2) [27]
Dyslipidemia, n (%)	27(62.8) [43]	14 (63.6) [22]	33 (61.1) [54]	8 (72.7) [11]	23 (60.5) [38]	18 (66.7) [27]
Cardiorenal syndrome type 1, n (%)	21(48.8) [43]	14 (63.6) [22]	25 (46.3) [54]	11(100.0) [11] ***	16 (42.1) [38]	19 (70.4) [27]*
Chronic kidney disease, n (%)	20(46.5) [43]	14 (63.6) [22]	25 (46.3) [54]	9 (81.8) [11] *	19 (50.0) [38]	15 (55.6) [27]
Ischemic heart disease, n (%)	13(30.2) [43]	9 (40.9) [22]	17 (31.5) [54]	5 (45.5) [11]	10 (26.3) [38]	12 (44.4) [27]
Valvular heart disease, n (%)	36(83.7) [43]	20 (90.9) [22]	46 (85.2) [54]	9 (81.8) [11]	32 (84.2) [38]	24 (88.9) [27]
Hypertensive heart disease, n (%)	27(62.8) [43]	17 (77.3) [22]	36 (66.7) [54]	7 (63.6) [11]	28 (73.7) [38]	16 (59.3) [27]

Hematological Parameters						
Hemoglobin, mean (SD), g/ dL	12.1 (1.8) [43]	10.9 (1.8) [22]*	11.9 (1.8) [54]	10.9 (1.9) [11]	12.0 (1.9) [38]	11.3 (1.8) [27]
MCV, median (Q1-Q3), fL	91.3 (85.7 - 94.5) [43]	91.3 (85.1 - 100.7) [22]	90.8 (85.2 - 96.3) [54]	92.0 (85.7 - 95.6) [11]	90.0 (84.3 - 95.4) [38]	92.5 (85.4 - 98.0) [27]
RDW, median (Q1-Q3), %	14.8 (13.8 - 15.4) [43]	16.1 (14.5 - 17.5) [22] *	14.9 (13.8 - 15.7) [54]	16.9 (15.7 - 17.6)[11] **	14.7 (13.8 - 15.7) [38]	15.4 (14.8 - 17.2) [27] *
Serum iron, median (Q1- Q3), µg/dL	42.1 (25.0 - 61.8) [42]	38.5 (29.0 - 48.4) [14]	41.8 (27.8 - 59.7) [48]	32.8 (22.7 - 61.8) [9]	35.6 (27.8 - 58.8) [35]	43.2 (22.8 - 61.4) [22]
Serum ferritin, median (Q1- Q3), ng/ml	212.7 (72.2 - 362.9) [39]	112.1 (52.3 - 243.4) [13]	212.7 (79.8 - 374.9) [45]	85.8 (52.3 - 95.8) [9]	153.9 (43.3 - 372.7) [32]	121.0 (64.7 - 299.8) [21]
Transferrin saturation, median (Q1- Q3), %	15.0 (10.0 - 21.0) [40]	13.5 (9.5 - 18.0) [14]	14.0 (10.0 - 21.0) [46]	19.0 (7.0 - 20.0) [9]	13.0 (10.0 - 21.0) [34]	15.0 (10.0 - 20.0) [21]
Total iron- binding capacity, median (Q1- Q3), µg/dL	281.8 (235.0 - 330.8) [40]	272.0 (218.0 - 293.8) [14]	275.0 (230.5 - 299.5) [46]	309.0 (224.6 - 336.0) [9]	278.0 (241.2 - 299.5) [34]	288.0 (224.6 - 336.0) [21]
EPO, median (Q1-Q3), mU/ mL	13.8 (10.3 - 18.2) [34]	20.2 (7.5 - 51.9) [15]	14.1 (9.6 - 20.2) [41]	39.4 (21.3 - 81.9) [6]*	11.9 (7.9 - 16.3) [28]	20.5 (15.7 - 46.5)[21]***

Anemia & iron deficiency						
Anemia, n (%)	20 (46.5) [43]	18 (81.8) [22]**	30 (55.6) [54]	8 (72.7) [11]	20 (52.6) [38]	18 (66.7) [27]
Iron deficiency, n (%)	20 (46.5) [39]	9 (40.9) [13]	23 (42.6) [45]	7 (63.6) [9]	17 (44.7) [32]	13 (48.1) [21]
Absolute iron deficiency, n (%)	13 (30.2) [39]	6 (27.3) [13]	13 (24.1) [45]	7 (63.6) [9] **	12 (31.6) [32]	8 (29.6) [21]
Functional iron deficiency, n (%)	7 (16.3) [39]	3 (13.6) [13]	10 (18.5) [45]	0 (0.0) [9]	5 (13.2) [32]	5 (18.5) [21]
Anemia with iron deficiency, n (%)	8 (18.6) [42]	8 (36.4) [16] *	11 (20.4) [50]	6 (54.5) [10] *	8 (21.1) [36]	9 (33.3) [23]
Anemia with absolute iron deficiency, n (%)	7 (16.3) [42]	6 (27.3) [16]	8 (14.8) [50]	6 (54.5) [10] **	7 (18.4) [36]	7 (25.9) [23]
Anemia with functional iron deficiency, n (%)	1 (2.3) [42]	2 (9.1) [16]	3 (5.6) [50]	0 (0.0) [10]	1 (2.6) [36]	2 (7.4) [23]
Anemia without iron deficiency, n (%)	11 (25.6) [42]	4 (18.2) [16]	15 (27.8) [50]	1 (9.1) [10]	10 (26.3) [36]	5 (18.5) [23]
Anemia without absolute iron deficiency, n (%)	12 (27.9) [42]	6 (27.3) [16]	18 (33.3) [50]	1 (9.1) [10]	11 (28.9) [36]	7 (25.9) [23]
Anemia without functional iron deficiency, n (%)	18 (41.9) [42]	10 (45.5) [16]	23 (42.6) [50]	7 (63.6) [10]	17 (44.7) [36]	12 (44.4) [23]
Iron deficiency without anemia, n (%)	12 (27.9) [39]	1 (4.5) [13]	12 (22.2) [45]	1 (9.1) [9]	9 (23.7) [32]	4 (14.8) [21]
Absolute iron deficiency without anemia, n (%)	6 (14.0) [39]	0 (0.0) [13]	5 (9.3) [45]	1 (9.1) [9]	5 (13.2) [32]	1 (3.7) [21]
Functional iron deficiency without anemia, n (%)	6 (14.0) [39]	1 (4.5) [13]	7 (13.0) [45]	0 (0.0) [9]	4 (10.5) [32]	3 (11.1) [21]

BMI, body mass index; EPO, erythropoietin; LVEF, left ventricular ejection fraction; MCV, mean corpuscular volume; NYHA, New York Heart Association; Q1, first quartile; Q3, third quartile; RDW, red blood cell distribution width.
Cardiorenal syndrome type I was defined as an acute worsening of cardiac function leading to acute kidney injury (defined by an increase in serum creatinine of ≥0.3 mg/dL) in accordance to the 7th Acute Disease Quality Initiative (ADQI) Consensus Conference. House AA, Anand I, Bellomo R, et al. Definition and classification of Cardio-Renal Syndromes: workgroup statements from the 7th ADQI Consensus Conference. Nephrol Dial Transplant. 2010 May; 25(5):1416-20.
N, number of subjects included in the study; [n], number of subjects with available data. *p-value*: * < 0.05; ** < 0.01; *** < 0.001

Table 3. Crude survival analysis for the study population

Characteristics	Short-term rehospitalization	Short-term mortality	End of follow-up mortality
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Age, years	0.99 (0.96-1.03)	1.02 (0.96-1.08)	0.98 (0.95-1.02)
Female	1.06 (0.46-2.47)	1.39 (0.41-4.76)	0.81 (0.38-1.73)
NYHA			
NYHA III	-	-	-
NYHA IV	0.94 (0.39-2.24)	0.82 (0.24-2.81)	0.53 (0.25-1.14)
LVEF, n (%)			
Preserved (> 49%)	-	-	-
Midrange (40-49%)	1.07 (0.34-3.41)	1.69 (0.28-10.12)	1.71 (0.67-4.36)
Reduced (< 40%)	1.29 (0.49-3.4)	2.05 (0.41-10.18)	1.42 (0.58-3.49)
Comorbidities			
Diabetes mellitus type 2	1.41 (0.61-3.27)	1.32 (0.40-4.31)	1.31 (0.61-2.8)
Obesity [BMI > 30 kg/m2]	1.51 (0.61-3.75)	0.40 (0.05-3.28)	0.54 (0.21-1.44)
Arterial hypertension	3.01 (0.4-22.39)	0.49 (0.11-2.27)	0.53 (0.18-1.53)
Dyslipidemia	1.08 (0.45-2.59)	1.64 (0.44-6.19)	1.28 (0.58-2.86)
Cardiorenal syndrome type 1	1.78 (0.75-4.25)	N/C	2.72 (1.19-6.23) *
Chronic kidney disease	1.86 (0.78-4.43)	4.63 (1.00-21.44)	1.35 (0.63-2.88)
Ischemic heart disease	1.32 (0.57-3.10)	1.78 (0.54-5.82)	1.84 (0.86-3.93)
Valvular heart disease	1.65 (0.39-7.08)	0.81 (0.18-3.76)	1.34 (0.4-4.46)
Hypertensive heart disease	1.82 (0.67-4.94)	0.88 (0.26-3.01)	0.66 (0.31-1.42)

Hematological parameters			
Hemoglobin, [per increments of 1 g/dL]	0.78 (0.64-0.96) *	0.77 (0.58-1.04)	0.85 (0.7-1.03)
RDW, [per increments of 10%] > 30 kg/m2]	1.35 (1.16-1.58) ***	1.83 (1.29-2.59) ***	1.31 (1.12-1.54) ***
Serum iron, [per increments of 10 ug/dL]hypertension	0.90 (0.7-1.16)	0.97 (0.72-1.32)	0.98 (0.8-1.19)
Serum ferritin, [per increments of 50 ng/ml]	0.93 (0.81-1.07)	0.82 (0.64-1.05)	0.97 (0.88-1.06)
Transferrin saturation, [per increments of 1%]	0.97 (0.9-1.04)	1.01 (0.92-1.1)	1.00 (0.94-1.06)
Total iron-binding capacity, [per increments of 10 µg/dL]	0.95 (0.88-1.02)	1.02 (0.93-1.1)	1.01 (0.95-1.07)
EPO, [per increments of 10 mU/ mL]	1.06 (0.99-1.14)	1.38 (1.04-1.82) *	1.29 (1.11-1.49) ***
Anemia & iron deficiency			
Anemia [female Hb <12 g/dL, male Hb <13 g/dL]	3.81 (1.29-11.28) *	1.98 (0.52-7.46)	1.56 (0.7-3.48)
Iron deficiency	2.10 (0.65-6.83)	3.13 (0.65-15.07)	1.40 (0.58-3.38)
Absolute iron deficiency	2.10 (0.7-6.26)	7.22 (1.5-34.81) *	1.19 (0.49-2.88)
Functional iron deficiency	1.03 (0.28-3.73)	N/C	1.28 (0.47-3.51)
Anemia with iron deficiency	3.50 (1.3-9.38) *	4.48 (1.26-15.88) *	1.91 (0.82-4.41)
Anemia with absolute iron deficiency	3.14 (1.13-8.74) *	6.14 (1.73-21.8) **	1.81 (0.74-4.4)
Anemia with functional iron deficiency	2.18 (0.5-9.62)¥	N/C¥	1.58 (0.37-6.75)¥
Anemia without iron deficiency	0.82 (0.27-2.56)	0.28 (0.03-2.18)	0.74 (0.28-2)
Anemia without absolute iron deficiency	1.12 (0.41-3.08)	0.21 (0.03-1.69)	0.89 (0.37-2.17)
Anemia without functional iron deficiency	2.04 (0.74-5.61)	2.48 (0.64-9.61)	1.25 (0.55-2.82)
Iron deficiency without anemia	0.21 (0.03-1.65)	0.38 (0.05-3.05)	0.68 (0.23-2.02)
Absolute iron deficiency without anemia	N/C¥	1.05 (0.13-8.38)¥	0.35 (0.05-2.64)¥
Functional iron deficiency without anemia	0.44 (0.06-3.38)¥	N/C¥	1.09 (0.32-3.69)¥

BMI, body mass index; CI, confidence interval; EPO, erythropoietin; Hb, hemoglobin; HR, hazards ratio; LVEF, left ventricular ejection fraction; N/C, not calculable because too small sample size; NYHA, New York Heart Association; RDW, red blood cell distribution width. p-value: * < 0.05; ** < 0.01; *** < 0.001

¥ Cases where number of events were less than 10.

We were able to confirm such high rates, as 58.5% of the study population had anemia and 46.2% had ID. Importantly, iron depletion in the setting of ADHF is predominantly absolute[15], as observed in our study.

We found that anemia was associated with an increased risk of short-term rehospitalization, a finding that is consistent with previously published studies.[8]

In addition to short-term rehospitalization, a connection between anemia and increased mortality in HF patients has been established in several studies[7,8], nevertheless, it is not unanimous if it is a predictor of mortality or simply a marker of more severe HF[18].

In our study, we found an association between anemia and an increased risk for end of follow-up mortality in the HFrEF subgroup. We also found an association with end of follow-up mortality for patients with anemia and ID.

Customarily, anemia results from depleted iron stores and/or impaired absorption or transport.[19] Anemia in HF is multifactorial and results from the combined effect of hemodilution, renal dysfunction, ID, resistance to EPO and chronic inflammation.[5,19]

Heart failure, *per se*, is known to promote an inflammatory response through cytokine production, which may lead to damage of the bone marrow and thereby provoking anemia.[20] Although the inflammatory role of elevated hepcidin in HF, which inhibits iron absorption, has been hypothesized, recent studies found a rather low-hepcidin profile in HF patients.[18] The mechanism by which anemia aggravates HF prognosis is not clear, although it seems to be linked to increased myocardial workload.[21]

The pathophysiological pathway for progressive ID in both chronic and acute HF also lacks clarification.[19] It has been postulated that HF patients may develop ID due to the depletion of iron stores or defective iron absorption and limited availability of iron recycled in the reticuloendothelial and monocyte-macrophage systems.[16]

It appears that HF contributes to myocardial iron depletion and, on the other hand, myocardial iron shortage aggravates HF, in a cyclic fashion.[22]

In our study, absolute ID was associated with short-term mortality and in the HFrEF subgroup elevated serum iron was associated with a reduced risk for short-term mortality.

Although ID has been considered to have clinical repercussion only in the presence of anemia, Klip et al recognized that ID was a robust independent predictor of mortality, irrespective of anemia, and that its predictive power could be superior to that of anemia. The EFFECT-HF study showed that it is advantageous to treat ID in both patients with and without anemia to relieve symptoms and improve exercise capacity and quality of life.[23]

Moreover, a meta-analysis evaluated randomized clinical trials that studied the effect of intravenous iron therapy in iron-deficient patients with HFrEF and suggested that beside the improvement in HF symptoms, exercise capacity and quality of life, the combined endpoint of mortality and hospitalizations was significantly reduced.[24]

The recently published AFFIRM-AHF trial acknowledged that the treatment of ID in patients admitted with ADHF reduced HF hospitalisations.[17]

Iron deficiency arises, not only as an independent prognostic marker, but also, as an interesting new treatment target for symptom relief in selected HF patients and eventually for reduction of hard-endpoints namely hospitalization and mortality.

RDW is a measure of anisocytosis, which is the variability in size of the circulating erythrocytes.[9] The major causes of anisocytosis are impaired erythrocyte production (such as deficiency of hematopoietic factors: iron, cobalamin and folate) and increased red cell destruction (namely hemolysis).[9] Several conditions known to compromise red cell balance (e.g. inflammatory distress, chronic renal failure, nutritional deficiencies, hepatic congestion) are common in patients with HF.[9] Therefore, although not specific, nor directly related to HF pathophysiology, this commonly overlooked parameter seems to identify worse morbid scenarios and therefore correlate with poor prognosis.[9]

Even though the physiological nexus between RDW and cardiovascular outcomes is not completely understood, some authors propose that oxidative stress and chronic subclinical inflammation resulting in dysregulation of iron homeostasis could be the cause.[25,26]

High baseline RDW has been considered an independent predictor of worse HF prognosis.[9] After analyzing data from the CHARM Program and the Duke Databank for Cardiovascular Diseases it was found that high RDW levels are associated with hospitalization and all-cause mortality in patients with HF.[9]

Another study also recognized RDW as a predictor of major adverse cardiac events.[27]

Our findings are in line with the previous studies, moreover, we extended the current knowledge by showing that RDW is also associated with short-term rehospitalization in HF patients.

We also found that EPO levels were associated with end of follow-up mortality in HF patients.

Belonje et al studied 605 HF patients and demonstrated that higher levels of EPO were linked with increased mortality.[10]

Nagai et al examined 539 ADHF patients and, also, acknowledged a link between elevated EPO levels and all-cause mortality.[28]

However, few studies have addressed the association between EPO levels and short- term mortality. To our knowledge, this is one of the first studies showing an association between increased EPO levels and risk for short-term mortality in HF patients.

Both EPO and RDW were associated with a higher risk for short-term death compared to end of follow-up mortality, which corroborates the assumption that patients with HF are specifically vulnerable during the first 90-days after discharge. This finding may have clinical implications and patients may need to be followed up more closely during this period.

EPO is a hormone produced in the juxtaglomerular cells of the kidney in response to local hypoxia (secondary to renal hypoperfusion) and/or systemic hypoxia (caused by HF, anemia, pulmonary disorders, or infectious diseases), which promotes erythropoiesis and ensures oxygen delivery to the tissue. Therefore, EPO is a marker of hypoperfusion, which is a paramount characteristic of the HF syndrome, hypoxia and inflammation and translates to disease severity.[29]

In addition, the bone marrow may become resistant to EPO due to the paucity of iron and other hematopoietic factors.[30] Low levels of iron are often seen in HF patients and could also play a role in the increased production of EPO.[30]

The complexity of these mechanisms suggests a weak correlation between EPO and hemoglobin values in CHF patients.[11] Consequently, although EPO is upregulated in anemic conditions, this relation is not linear in patients with CHF. Such finding may justify the benefit of correcting hematopoietic factors deficit (namely ID) in non-anemic patients.

Our study has several potential limitations that must be taken into consideration.

Due to the small sample size and, therefore, the small number of events of interest, it was decided not to perform a multivariable analysis. Furthermore, our study was a single-center study which may limit the ability to generalize our results.

As far as treatment is concerned all patients were treated in accordance to the ESC guidelines.

Despite these potential concerns, our study reports data from a real-world clinical setting and the results are consistent with previously published studies, hence supporting the validity of our findings.

CONCLUSIONS

Anemia and RDW correlated with higher risk for short-term rehospitalization.

Absolute iron deficiency, RDW and EPO were associated with higher risk for short- term mortality.

RDW and EPO were associated with higher risk for end of follow-up mortality.

Our findings may provide insight into HF prognosis and raise the interest in some neglected hematological parameters.

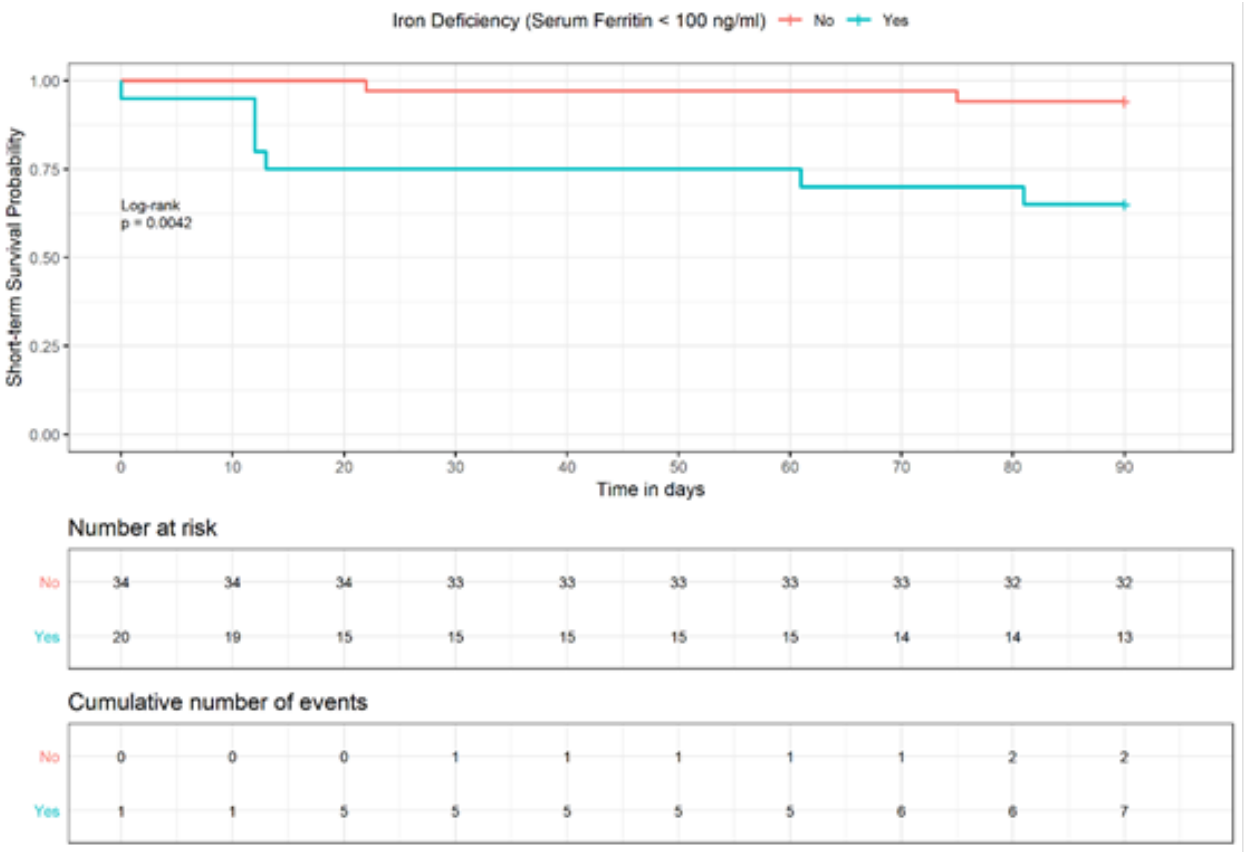
These parameters can be measured in most clinical settings and may be a valuable tool for risk stratification of ADHF patients.

Supplementary Table 1 - Survival analysis according to LVEF

Characteristics	Short-term rehospitalization	Short-term mortality	End of follow-up mortality
	HR (95% CI)	HR (95% CI)	HR (95% CI)
HFpEF			
Serum iron, [per increments of 10], ug/dL	0.91 (0.55-1.50)	1.06 (0.49-2.28)	1.03 (0.69-1.52)
EPO, [per increments of 10], mU/mL	0.76 (0.31-1.88)	1.41 (0.83-2.38)	1.30 (0.93-1.80)
Anemia [female Hb <12 g/dL, male Hb <13 g/dL]	1.75 (0.45-6.78)	0.41 (0.04-4.51)	0.68 (0.22-2.10)
HFmrEF			
Serum iron, [per increments of 10], ug/dL	0.33 (0.05-2.20)	3.33 (0.51-21.67)	1.49 (0.99-2.24)
EPO, [per increments of 10], mU/mL	1.42 (0.82-2.46)	1.09 (0.42-2.81)	0.82 (0.50-1.34)
Anemia [female Hb <12 g/dL, male Hb <13 g/dL]	N/C	0.27 (0.02-4.41)	0.70 (0.14-3.64)
HFrEF			
Serum iron, [per increments of 10], ug/dL	0.96 (0.64-1.44)	0.34 (0.12-0.98) *	0.70 (0.45-1.11)
EPO, [per increments of 10], mU/mL	1.08 (0.99-1.19)	N/C	1.81 (1.14-2.88) *
Anemia [female Hb <12 g/dL, male Hb <13 g/dL]	9.18 (1.08-77.88)	N/C	11.14 (1.35-92.07)*

CI, confidence interval; EPO, erythropoietin; HFmrEF, heart failure with midrange ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HR, hazards ratio; N/C, not calculable because too small sample size.
p-value: * < 0.05

Figure 2. Kaplan Meier survival curves for short-term mortality rate according to absolute iron deficiency status



CONFLICT OF INTEREST

The authors state they have given presentations sponsored by Laboratorios Nutrición Medica SL.

SOURCE OF FUNDING

None to declare.

ETHICAL ASPECTS

All patients gave written informed consent. The study was approved by an Institutional Review Board (Academic Medical Center Ethics Committee) and was conducted in accordance with the Declaration of Helsinki and the Oviedo Convention.

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