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RESEARCH ARTICLE

Iron deficiency contributes to resistance to endogenous erythropoietin in anaemic heart failure patients

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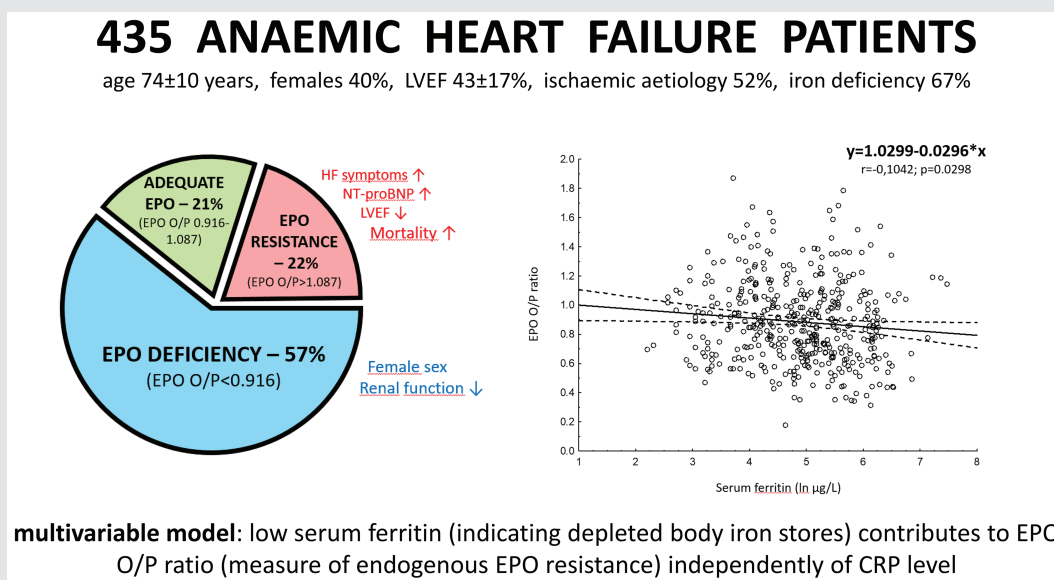|	
Aims	Abnormal endogenous erythropoietin (EPO) constitutes an important cause of anaemia in chronic diseases. We analysed the relationships between iron deficiency (ID) and the adequacy of endogenous EPO in anaemic heart failure (HF) patients, and the impact of abnormal EPO on 12-month mortality.
Methods and results	We investigated 435 anaemic HF patients (age: 74 ± 10 years; males: 60%; New York Heart Association class I or II: 39%; left ventricular ejection fraction: 43 ± 17%). Patients with EPO higher than expected for a given haemoglobin were considered EPO-resistant whereas those with EPO lower than expected - EPO-deficient. ID was defined as serum ferritin $<100\,\mu g/L$ or $100-299\,\mu g/L$ with transferrin saturation <20%. EPO-resistant patients (22%) had more advanced HF whereas those with EPO deficiency (57%) were more frequently females and had worse renal function. Lower serum ferritin (indicating depleted body iron stores) was related to higher EPO observed/predicted ratio when adjusted for significant clinical confounders, including C-reactive protein. One year all-cause mortality was 28% in patients with EPO resistance compared to 17% in patients with EPO deficiency and 10% in patients with adequate EPO (log-rank test for the comparison EPO resistance vs. adequate EPO: $P = 0.02$). When adjusted for other prognosticators, there was still a trend towards increased 12-month mortality in patients with higher EPO level.
Conclusion	Anaemic HF patients with endogenous EPO deficiency vs. resistance have different clinical and laboratory characteristics. In such patients, ID contributes to EPO resistance independently of inflammation.

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Graphical Abstract

435 ANAEMIC HEART FAILURE PATIENTS
age 74±10 years, females 40%, LVEF 43±17%, ischaemic aetiology 52%, iron deficiency 67%

multivariable model: low serum ferritin (indicating depleted body iron stores) contributes to EPO O/P ratio (measure of endogenous EPO resistance) independently of CRP level

The majority of anaemic HF patients have abnormal endogenous erythropoietin (EPO) level, and patients with endogenous EPO deficiency versus resistance have different clinical and laboratory characteristics. In such patients, depleted body iron stores contribute to the resistance to endogenous EPO independently of inflammation.

Introduction

Absolute or functional deficiency of endogenous erythropoietin (EPO) constitutes an important cause of anaemia in different chronic diseases.[1,2] For example, in advanced chronic kidney disease (CKD) many patients either develop an absolute EPO deficiency due to its decreased renal production, or present with resistance to endogenous EPO as the result of a chronic pro-inflammatory state.[3] Importantly, in the haematology literature, patients with anaemia due to iron deficiency (ID) have comparable haemoglobin concentration but higher circulating EPO than patients with anaemia of chronic disease, which suggests the contribution of ID to endogenous EPO resistance.[4] In anaemic heart failure (HF) patients, quantitative and qualitative derangements regarding endogenous EPO contribute to the development and progression of anaemia; however, it is still unclear whether particular pathomechanisms known from other chronic conditions such as CKD are also valid in the HF population.[5]

Hence, in the current study we aimed to investigate: (i) the prevalence of endogenous EPO deficiency and resistance in anaemic HF patients, (ii) the relationships between inflammation, ID and EPO level in these patients, and (iii) the impact of abnormal EPO on 12-month mortality.

Methods

Patients

The baseline study population comprised 1821 patients with stable, chronic HF with a broad range of left ventricular ejection fraction (mean ± standard deviation: 35 ± 15%, median: 32%)[6] from five cohorts from: (i) Poland (two cohorts, $n = 735$),[7,8] (ii) Spain (one cohort, $n = 789$),[9] and (iii) the Netherlands (two cohorts, $n = 297$)[10,11] as described previously. Detailed inclusion and exclusion criteria for each cohort are available as an online Appendix to the primary paper.[6] Of the original international pooled cohort including 1821 patients, 587 enrolees (32%) were anaemic (haemoglobin <13.0 g/dL in men and <12.0 g/dL in women, based on World Health Organization definition),[12] which prevalence is comparable to one large systematic review on anaemia in chronic HF.[13] Here we report on 435 HF patients with anaemia who had endogenous EPO measured at enrolment, which constitute 74% of all anaemic subjects. At the time of the inclusion in the study (during the outpatient visit or at elective hospitalization), none of the patients recruited were treated with erythropoiesis-stimulating agents (ESA) or intravenous iron therapy, and neither had blood transfusions. All study protocols were approved by the local ethics committees, and all patients gave written informed consent. The study was conducted in accordance with the Helsinki Declaration.

Erythropoietin, haematological parameters, iron status, and other laboratory tests

All laboratory tests were measured only at baseline. Laboratory measurements were performed locally at each centre. After centrifuging, some plasma and serum were collected and frozen for further analyses. Endogenous EPO was measured using an immunoassay. Haemoglobin and red cell indices were measured in fresh venous blood. The following iron parameters 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. Serum iron and TIBC were assessed using a substrate method. Transferrin saturation (TSAT) was calculated as a ratio of $0.7217 \times$ serum iron and transferrin, multiplied by 100.[14] When transferrin was not available, TSAT was calculated as a ratio of serum iron and TIBC, multiplied by 100. ID was defined based on large randomized controlled trials on ID in HF as serum ferritin <100 µg/L (absolute ID) or serum ferritin 100−299 µg/L in combination with a TSAT <20% (functional ID).[15,16] Serum soluble transferrin receptor (sTfR) was measured using immunonephelometry or an enzyme immunoassay. Patients' renal function was assessed using estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease equation.[17] C-reactive protein (CRP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were measured using conventional methods.

Adequacy of endogenous erythropoietin

The adequacy of endogenous EPO in investigated anaemic HF patients was evaluated using the observed/predicted (O/P) ratio as previously described.[5] EPO O/P ratio was calculated as follows: $\log_{10}$(EPO observed)/$\log_{10}$(EPO predicted). Predicted concentration of EPO was calculated with the following formula: $\log_{10}$(EPO) = 4.640 − [0.274*haemoglobin].[5] Patients with EPO higher than expected for a given haemoglobin (EPO O/P ratio >1.087) were considered EPO-resistant, whereas those with EPO levels lower than expected (EPO O/P ratio <0.916) were classified as EPO-deficient.[5]

Statistical analyses

Most continuous variables had a normal distribution, and were expressed as a mean ± standard deviation. The inter-group differences were tested using the analysis of variance (ANOVA) with post-hoc Scheffé's tests. NT-proBNP, eGFR, CRP, ferritin, and EPO had a skewed distribution, and were log-transformed (a natural logarithm, ln) before the inclusion in further analyses. These variables were expressed as median with interquartile range (IQR), and the inter-group differences were tested after ln-transformation. Categorized variables were expressed as number and proportion (%), and the inter-group differences were tested using the Chi-square test. Univariable logistic regression analyses were applied to establish the variables associated with the higher prevalence of EPO resistance or EPO deficiency (both vs. adequate EPO). As potential risk factors for the greater prevalence of inadequate EPO, we analysed variables describing the severity and aetiology of HF, comorbidities, iron status, red cell indices, and other laboratory parameters.

Univariable and multivariable linear regression analyses were applied to establish variables associated with EPO O/P ratio. In univariable analyses, as potential correlates of EPO O/P ratio, we included parameters describing the severity and aetiology of HF, comorbidities, iron status,

red cell indices, and other laboratory parameters. During the construction of multivariable models, we included significant ($P < 0.05$) determinants of EPO O/P ratio from univariable analyses.

The associations between analysed variables related to the adequacy of endogenous EPO with 12-month mortality were evaluated using univariable and multivariable Cox proportional hazard regression models. The impact of endogenous EPO level (ln), EPO O/P and abnormal (higher or lower than expected) vs. adequate EPO on 12-month mortality was tested alone (univariable models) and after adjusting for other significant prognosticators from univariable analyses. We also verified whether the impact of endogenous EPO on 12-month mortality may be U-shaped using the natural cubic splines methodology. Additionally, we constructed Kaplan−Meier curves to compare survival in patients with EPO resistance or deficiency vs. adequate EPO. Twelve-month mortality between these three groups of patients was compared using log-rank tests. The length of follow-up of survivors and patients who died later than 12 months after enrolment was censored at 365 days.

A P-value of <0.05 was considered statistically significant. Statistical analyses were performed using the Statistica 13.3 data analysis software system (TIBCO, Inc).

Results

Prevalence and clinical correlates of abnormal endogenous erythropoietin

Baseline clinical characteristics of examined patients with HF are presented in *Table 1*. Overall, 22% of patients were EPO-resistant, 57% EPO-deficient, and only 21% had an adequate EPO level. Patients with EPO resistance compared with those with adequate EPO were characterized by more severe HF symptoms, lower left ventricular ejection fraction (LVEF), higher NT-proBNP, and slightly higher haemoglobin (*Table 1*, *Figure 1*). Patients with EPO deficiency (vs. those with EPO within normal range) were more frequently female and had worse renal function (*Table 1*, *Figure 1*). Among 435 analysed anaemic HF patients, 292 subjects were iron-deficient (67%), including 122 with functional and 170 with absolute ID (28% and 39%, respectively). Patients with absolute ID had a median EPO of 25 U/L (IQR 16−42), with functional ID 19 U/L (12−44), and subjects without ID 19 U/L (11−30) [P-value for ANOVA =0.001 and P-value for post-hoc comparison between absolute ID vs. no ID =0.001; the other two post-hoc comparisons (functional vs. absolute ID and no ID vs. functional ID) were not significant]. In the group of investigated patients with HF as much as 77% were receiving angiotensin-converting enzyme inhibitor or angiotensin receptor blocker at enrolment, 86% beta-blocker, 41% aldosterone antagonist, and 91% loop diuretic. EPO level (ln) did not correlate with the use of aforementioned key groups of HF drugs (all $P > 0.2$). EPO O/P ratio was greater in subjects taking aldosterone antagonist ($r = 0.14$, $P = 0.006$) but this observation was not valid for other aforementioned groups of drugs (all $P > 0.6$).

Associations between iron status and abnormal erythropoietin

In multivariable linear regression models, concomitant ID as well as lower serum ferritin were related to higher EPO O/P

Table 1 Baseline characteristics of anaemic heart failure patients according to the adequacy of endogenous erythropoietin (EPO). Clinical and laboratory correlates of EPO resistance and EPO deficiency (univariable logistic regression analyses)

Variable	N	Baseline characteristics and intergroup comparisons					Univariable logistic regression analyses OR (95% CI) χ^2		
		All patients with HF (n = 435)	Patients with EPO within expected range (n = 90) [EPO O/P: 0.916–1.087]	Patients with EPO higher than expected (n = 95) [EPO O/P > 1.087][a]	Patients with EPO lower than expected (n = 250) [EPO O/P < 0.916][a]	P-value for ANOVA/χ^2 test	Unit	Prediction of EPO resistance (EPO higher than expected vs. normal EPO)	Prediction of EPO deficiency (EPO lower than expected vs. normal EPO)
Age (years)	435	74 ± 10	72 ± 10	73 ± 10	74 ± 10	0.377	Year	–	–
Male sex	435	261 (60%)	62 (69%)	76 (80%)	123 (49%)**	<0.001	Male vs. female	–	0.44 (0.26–0.73) 10.1**
BMI (kg/m²)	410	27.9 ± 5.6	28.5 ± 6.3	27.4 ± 4.6	27.9 ± 5.6	0.474	kg/m²	–	–
NYHA class (I–II/III –IV)	435	169/266 (39/61%)	37/53 (41/59%)	24/71 (25/75%)*	108/142 (43/57%)	0.008	III–IV vs. I–II	2.07 (1.10–3.87) 5.17*	–
HF aetiology, ischaemic	435	225 (52%)	50 (56%)	61 (64%)	114 (46%)	0.006	Ischaemic vs. non-ischaemic	–	–
LVEF (%)	435	43 ± 17	43 ± 16	37 ± 17**	45 ± 17	<0.001	%	0.98 (0.96–0.99) 6.87**	–
NT-proBNP (pg/mL)	429	2274 (948–5210)	2223 (1118–4874)	3670 (1375–7662)*	1959 (818–4599)	0.002	ln pg/mL	1.31 (1.03–1.66) 5.11*	–
eGFR (mL/min/1.73 m²)	435	73 (47–119)	84 (60–132)	75 (47–121)	68 (43–113)*	0.047	ln mL/min/1.73 m²	–	0.61 (0.41–0.90) 6.22*
CRP (mg/L)	381	1.3 (0.4–3.8)	1.0 (0.4–3.4)	1.4 (0.4–4.5)	1.3 (0.4–3.8)	0.188	ln mg/L	–	–
Comorbidities									
Arterial hypertension, yes	432	318 (74%)	62 (70%)	65 (68%)	191 (77%)	0.173	Yes vs. no	–	–
Diabetes, yes	426	222 (52%)	44 (50%)	47 (50%)	131 (54%)	0.753	Yes vs. no	–	–
Atrial fibrillation, yes	410	106 (26%)	25 (30%)	27 (30%)	54 (23%)	0.227	Yes vs. no	–	–
Iron status									
Iron deficiency, yes	435	292 (67%)	64 (71%)	69 (73%)	159 (64%)	0.186	Yes vs. no	–	–
Iron (µg/dL)	435	52 ± 33	51 ± 28	52 ± 26	53 ± 36	0.946	µg/dL	–	–
Ferritin (µg/L)	435	139 (61–261)	119 (51–233)	106 (56–252)	151 (72–289)	0.111	ln µg/L	–	–
TSAT (%)	435	16 ± 10	15 ± 8	16 ± 9	16 ± 11	0.539	%	–	–
sTfR (mg/L)	270	1.93 ± 1.01	1.93 ± 0.84	2.33 ± 1.15*	1.83 ± 1.01	0.013	mg/L	–	–
Red cell indices and EPO									
Haemoglobin (g/dL)	435	11.2 ± 1.2	11.4 ± 1.0	12.0 ± 0.8***	10.7 ± 1.1***	<0.001	g/dL	1.98 (1.40–2.79) 15.3***	0.54 (0.41–0.69) 22.4***
MCV (fL)	378	90 ± 7	91 ± 7	89 ± 7	89 ± 7	0.259	fL	–	–
MCH (pg)	365	28 ± 3	29 ± 3	29 ± 3	28 ± 3	0.632	pg	–	–
MCHC (g/dL)	365	32 ± 1	32 ± 1	32 ± 2	32 ± 1	0.230	g/dL	–	–
EPO (U/L)	435	21.0 (12.0–37.0)	28.6 (19.0–47.7)	50 (31–81)***	14.0 (9.0–22.0)***	<0.001	–	–	–

Data are presented as mean ± standard deviation, median (with lower and upper quartile), or n (%), as appropriate.

Iron deficiency was defined as ferritin <100 µg/L, or 100–299 µg/L with TSAT <20%.

BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; HF, heart failure; ln, natural logarithm; 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; O/P, observed/predicted ratio; OR, odds ratio; sTfR, soluble transferrin receptor; TSAT, transferrin saturation.

[a]P-values are presented for the comparison with the group 'EPO within expected range' (post-hoc).

*P < 0.05.

**P < 0.01.

***P < 0.001.

ratio when adjusted for relevant clinical and laboratory confounders, including CRP (*Table 2*). The relationship between serum ferritin (ln) and EPO O/P ratio (linear regression slope) is shown in *Figure 2*. In a multivariable model comprising sTfR as an indicator of iron status, this parameter did not reach pre-defined level of statistical significance as an independent correlate of endogenous EPO O/P ratio ($\beta = 0.028$, $P = 0.076$) (*Table 2*).

Adequacy of endogenous erythropoietin and 12-month all-cause mortality

Follow-up was available for 283 patients and its median duration was 501 days (range: 1−3775). Median time to death (in 107 patients with reported death) was 468 days (range: 1−3314). Only 26 survivors had the duration of follow-up <365 days (one patient: 31 days; remaining 25 enrolees: 112−364 days). There was no difference regarding 12-month all-cause mortality in EPO-deficient patients (17%) vs. adequate EPO group (10%; log-rank test: $P = 0.23$), whereas EPO-resistant patients had higher mortality (28%) as compared with adequate EPO (log-rank: $P = 0.02$), and there was also a trend towards greater mortality in patients with EPO resistance vs. EPO deficiency (log-rank: $P = 0.06$) (*Figure 3*). When adjusted for relevant prognosticators from univariable analyses (i.e. HF severity and neurohormonal activation), there was still a trend towards higher 12-month mortality in patients with higher EPO (*Table 3*). To verify the potential U-shaped relationship between endogenous EPO and survival, we applied the natural cubic splines with five knots, but it did not influence significantly the 12-month all-cause mortality in investigated patients with HF and anaemia (EPO included as a cubic spline in the multivariable model: $P = 0.28$) (*Figure 4*).

Discussion

There are two major findings arising from our observational study. First, clinical and laboratory characteristics were different between patients with endogenous EPO deficiency and resistance, compared to those with adequate EPO levels. Second, ID contributed to endogenous EPO resistance but not EPO deficiency.

Heart failure and renal dysfunction frequently coexist and the pathophysiological concept of a 'cardio-renal anaemia syndrome' has been widely discussed in the literature.[18,19] Importantly, although HF and CKD share some pathophysiological phenomena related to erythropoiesis,[20,21] the mechanisms leading to anaemia in these two conditions are not entirely analogous.[20] Abnormal endogenous EPO has been previously shown to contribute to anaemia in HF.[22] In the study of van der Meer *et al.*[5] out of 74 chronic HF patients with concomitant anaemia, 29 were EPO-deficient and 22 EPO-resistant, and EPO O/P ratio correlated with CRP (r = 0.3, $P = 0.02$). Indeed, chronic pro-inflammatory state with excess of circulating cytokines, occurring in various chronic disorders, impedes erythropoiesis within the bone marrow which is orchestrated by endogenous EPO.[23] Although chronic low-grade inflammation (with the retention of iron in

mononuclear phagocyte system as one of its pathophysiological features) has been considered the key mechanism of EPO resistance in HF patients, data from haematological literature led us to hypothesize that also depleted iron stores may play a role in hampering pro-erythropoietic effects of endogenous EPO. For example, Theurl *et al.*[4] measured endogenous EPO levels in patients with anaemia of different aetiology, and demonstrated that subjects with iron-deficient anaemia have comparable haemoglobin but higher EPO, compared to the group with anaemia of chronic disease (recently 'anaemia of inflammation'). In the course of HF, one more mechanism may be responsible for a blunted response of the bone marrow to increasing endogenous EPO. Namely, the adaptive increase in the production of erythrocytes (as a response to progressive anaemia) is precisely orchestrated by the group of transcription factors called hypoxia-inducible factors (HIFs), which exert their pro-erythropoietic effects in a multidimensional way.[24] It needs to be acknowledged that HIFs not only stimulate renal (and liver) production of EPO, but also affect the microenvironment of the bone marrow, preparing it for increased proliferation of red blood cells.[24] There is experimental evidence from animal HF models that HIF signalling is abnormal in the course of cardiac volume overload.[25] One may ask the question if in the course of HF the bone marrow response to HIFs is as effective as that of the kidneys, irrespective of overlapping immune-driven mechanisms that were described above. The complex interplay between HIF signalling, chronic low-grade inflammation, dysregulated iron status, and anaemia in the course of HF requires further mechanistic research.

In our study we have shown that in anaemic HF patients, concomitant ID is related to higher EPO O/P ratio when adjusted for patient sex, HF severity, neurohormonal activation, renal function, and inflammation. Importantly, this relationship was driven predominantly by the depletion of iron stores, as reflected by lower serum ferritin. Analogously, circulating EPO level was comparably lower in subjects with functional ID and without ID (median 19 U/L for both groups), compared with subjects with absolute ID who had significantly greater EPO (median 25 U/L). This observation is another argument supporting the hypothesis that in anaemic HF patients without severe inflammation (such as for example in end-stage renal disease with uraemia) an absolute decrease in body iron (predominantly due to impaired intestinal iron absorption?) inhibits the actions of disproportionally elevated EPO to a greater extent than the immune-driven retention of iron in the mononuclear phagocyte system. The latter retention is the key element of anaemia of inflammation, in the course of which body iron stores do not have to be reduced.[26] Median CRP for our study group (1.3 mg/L) confirms that inflammatory processes were not severe in examined patients, comparing for example with the population of haemodialysed patients with end-stage renal disease. Our results indicate that mechanisms of EPO resistance related to absolute ID compared with inflammation-related functional ID may not be the same. Importantly, we have shown that ID can contribute to endogenous EPO resistance in anaemic HF patients in a broad spectrum of LVEF. Indeed, although ID complicating HF has been initially investigated (and treated) in subjects with lower values of LVEF,[15,16] now we have data that it is also common in HF with mid-range and
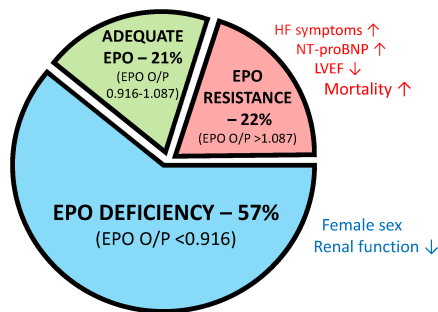
Figure 1 Prevalence and clinical correlates of abnormal endogenous erythropoietin (EPO) in anaemic heart failure (HF) patients. LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro B-type natriuretic peptide; O/P, observed/predicted.

preserved LVEF, and detrimental clinical effects of ID (decreased exercise capacity and worse outcomes) are evident across all LVEF categories.[27]

Importantly, our study group is also unique because all patients had concomitant anaemia. It needs to be acknowledged that 'universal' HF prognosticators – i.e. identified for all HF patients irrespective of the anaemia status – should not be simply extrapolated to anaemic subjects, who themselves have worse prognosis.[13,28] For example, in our cohort, NT-proBNP was not an independent predictor of increased 12-month mortality in the multivariable model. This may be due to the fact that anaemia itself affects circulating natriuretic peptides by increasing plasma volume, which effect is independent of current degree of HF-related congestion.[29,30] On the other hand, in the sub-analysis of the RED-HF clinical trial (see below) the prognostic value of

Table 2 Associations between endogenous erythropoietin observed/predicted ratio and clinical variables, comorbidities, iron status, and red cell indices in anaemic heart failure patients

Correlates of EPO O/P ratio (units)	Univariable models	Multivariable model with ID (corrected $R^2 = 13\%$, $P < 0.001$)	Multivariable model with ferritin (corrected $R^2 = 14\%$, $P < 0.001$)	Multivariable model with sTfR (corrected $R^2 = 13\%$, $P < 0.001$)
Age (years)	−0.002[#]			
Sex (male vs. female)	0.156***	0.115***	0.118***	0.071*
BMI (kg/m^2)	0.002			
NYHA class (III–IV vs. I–II)	0.074**	0.050[#]	0.050[#]	0.021
HF aetiology (ischaemic vs. non-ischaemic)	0.098***	0.040	0.041	0.031
LVEF (%)	−0.004***	−0.001	−0.001	0.000
NT-proBNP (ln pg/mL)	0.040***	0.027*	0.029**	0.055***
eGFR (ln mL/min/1.73 m^2)	0.043*	0.068**	0.070**	0.085**
CRP (ln mg/L)	0.032*	0.009	0.015	−0.053*
Comorbidities				
Arterial hypertension (yes vs. no)	−0.060[#]			
Diabetes (yes vs. no)	−0.007			
Atrial fibrillation (yes vs. no)	0.047			
Iron status				
Iron deficiency (yes vs. no)	0.069*	0.059*		
Iron (µg/dL)	−0.000			
Ferritin (ln µg/L)	−0.030*		−0.038**	
TSAT (%)	−0.002			
sTfR (mg/L)	0.052**			0.028[#]
Red cell indices				
MCV (fL)	0.002			
MCH (pg)	0.006			
MCHC (g/dL)	0.012			

Data are presented as regression coefficients β (both in univariable and multivariable models) between erythropoietin observed/predicted ratio (EPO O/P, dependent variable in the multivariable model) and other variable/variables.

BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HF, heart failure; ln, natural logarithm; 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; sTfR, soluble transferrin receptor; TSAT, transferrin saturation.

[#]$P < 0.1$ (trend).
*$P < 0.05$.
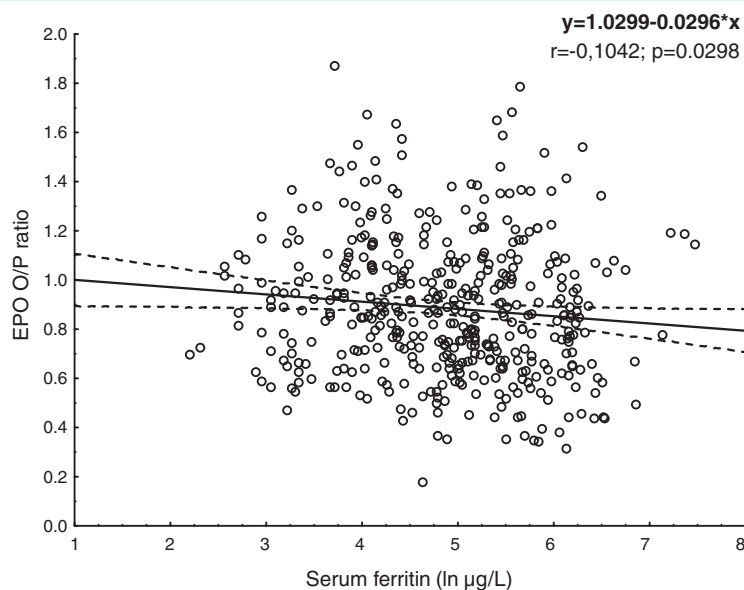**$P < 0.01$.
***$P < 0.001$.

Figure 2 Relationship between endogenous erythropoietin observed/predicted (EPO O/P) ratio and a natural logarithm of serum ferritin in patients with heart failure and anaemia [linear regression slope (solid line) with 95% confidence interval range (dashed lines)].
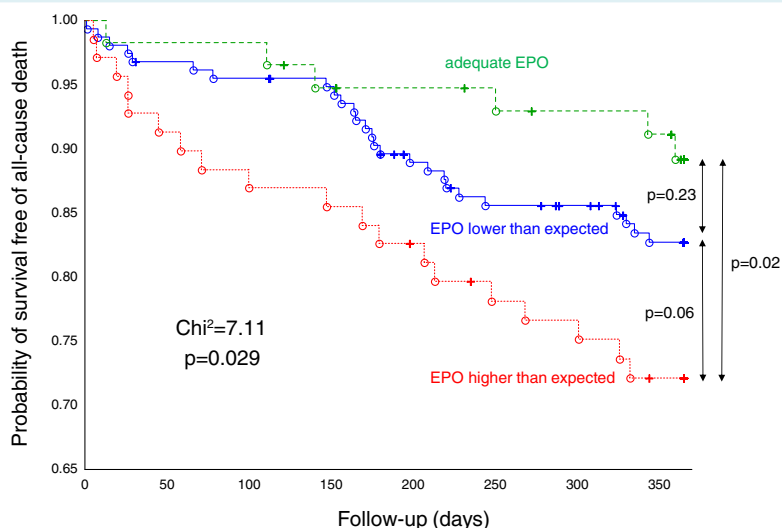

Figure 3 Kaplan–Meier survival curves for 12-month survival free of all-cause death for the three different groups of anaemic heart failure patients according to the adequacy of endogenous erythropoietin (EPO) level. Censored observations are marked with a cross and uncensored with a circle. *P*-values for the two-group comparisons are presented for the log-rank test, and for the three-group comparison there are presented the results for the Chi-square test.

NT-proBNP outperformed other analysed biomarkers for predicting adverse cardiac outcomes in chronic systolic HF patients with mild or moderate anaemia.[31] Nevertheless, the results of our study underline the differences between anaemic subjects and the general population of HF patients.

We have also demonstrated that advanced age, more severe HF symptoms and worse renal function were independent predictors of increased 12-month mortality, and there was a trend towards higher mortality in subjects with higher endogenous EPO. The latter trend for worse prognosis in subjects with elevated EPO is consistent with previous findings demonstrating significant clinical burden related to EPO resistance in anaemic HF patients.[5] One may hypothesize that endogenous EPO resistance is not a separate pathology but only a bystander in the progression of HF, as EPO O/P ratio was indeed higher in patients with more advanced cardiac disease. However, multivariable prognostic models included

Table 3 Prognosticators of 12-month all-cause mortality in patients with heart failure and anaemia (Cox proportional hazard regression models)

Prognosticator (unit)	Univariable models				Multivariable model $n = 239, \chi^2 = 45.4, P < 0.001$			
	HR	95% CI	χ^2	*P*-value	HR	95% CI	χ^2	*P*-value
Age (years)	1.05	1.02−1.09	9.34	0.002	1.07	1.02−1.11	8.88	0.003
Sex (male vs. female)	1.33	0.73−2.39	0.87	0.351				
BMI (kg/m^2)	0.96	0.91−1.02	1.84	0.175				
NYHA class (III−IV vs. I−II)	3.28	1.40−7.69	7.47	0.006	3.31	1.14−9.60	4.85	0.027
HF aetiology (ischaemic vs. non-ischaemic)	1.73	0.97−3.10	3.40	0.065				
LVEF (%)	0.99	0.97−1.00	2.11	0.146				
NT-proBNP (ln pg/mL)	1.64	1.30−2.08	17.3	<0.001	1.12	0.81−1.54	0.47	0.495
eGFR (ln mL/min/1.73 m^2)	0.31	0.19−0.50	22.2	<0.001	0.40	0.21−0.77	7.63	0.006
CRP (ln mg/L)	1.40	1.08−1.81	6.62	0.010	1.05	0.80−1.38	0.14	0.707
Comorbidities								
Arterial hypertension (yes vs. no)	0.86	0.48−1.53	0.27	0.606				
Diabetes (yes vs. no)	1.12	0.64−1.95	0.15	0.700				
Atrial fibrillation (yes vs. no)	0.72	0.35−1.49	0.80	0.372				
Iron status								
Iron deficiency (yes vs. no)	0.86	0.49−1.53	0.25	0.616				
Iron (μg/dL)	0.99	0.98−1.00	1.57	0.210				
Ferritin (ln μg/L)	1.37	1.02−1.83	4.50	0.034	1.32	0.95−1.84	2.74	0.098
TSAT (%)	0.97	0.94−1.01	2.48	0.115				
sTfR (mg/L)	0.99	0.72−1.37	0.01	0.967				
Red cell indices and EPO adequacy								
Haemoglobin (g/dL)	0.80	0.64−1.01	3.66	0.056				
MCV (fL)	1.01	0.97−1.06	0.21	0.648				
MCH (pg)	0.99	0.88−1.11	0.05	0.832				
MCHC (g/dL)	0.92	0.75−1.13	0.66	0.417				
EPO (ln U/L)	1.60	1.16−2.19	8.45	0.004	1.46	0.97−2.21	3.24	0.072
EPO O/P (ratio)	2.16	0.84−5.56	2.53	0.112				
EPO higher than expected vs. normal	2.95	1.18−7.38	5.33	0.021				
EPO lower than expected vs. normal	1.69	0.70−4.11	1.35	0.245				

BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; EPO, erythropoietin; HF, heart failure; HR, hazard ratio; ln, natural logarithm; 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; O/P, observed/predicted ratio; sTfR, soluble transferrin receptor; TSAT, transferrin saturation.

well-established HF prognosticators and the trend for the EPO was still present, in contrast to e.g. NT-proBNP which was mentioned above. Our study positions disproportionally elevated EPO as one of the elements of the pathophysiology of anaemia in HF, and further observational and interventional studies are needed to address the question whether endogenous EPO could be normalized in this particular patient group. In nephrology, the phenomenon of resistance to ESA in some patients with CKD is widely known, and addressing this clinical issue still remains challenging.[32] In such patients depleted iron stores due to e.g. poor nutrition or blood losses are a well recognized cause of ESA hyporesponsiveness (independent of inflammation), and intravenous iron is administered in such patients for the 'conditioning' of ESA therapy.[33] In the previously mentioned randomized, double-blind

RED-HF trial investigating the effects of ESA on morbidity and mortality in patients with systolic HF (the therapy was not effective), the median serum ferritin was 102 μg/L, suggesting that almost half of enrolees had absolute ID at the inclusion.[34,35] One may hypothesize that depleted iron may trigger the resistance to exogenous ESA in HF analogously to CKD. On the other hand, there is a sub-analysis of this trial demonstrating that baseline iron parameters were not related to the hyporesponsiveness to darbepoetin alfa.[36] Indisputably the pathophysiology of abnormal EPO responses in anaemic HF patients requires further research.

Study limitations

This study has certain limitations that should be emphasized. Firstly, of 587 anaemic patients from the original pooled cohort only 74%
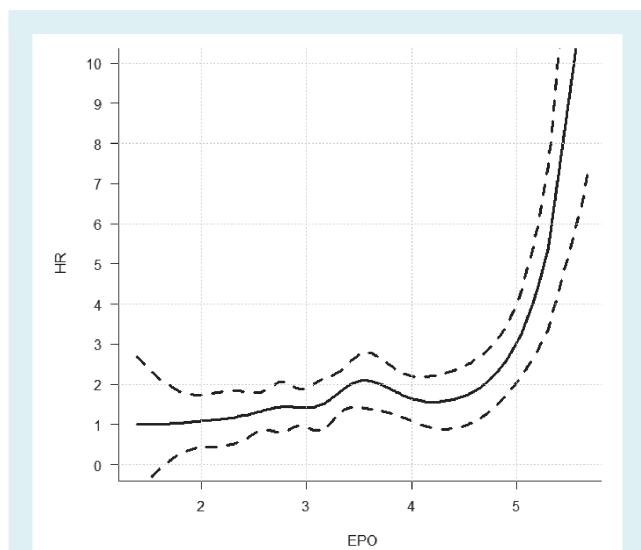
Figure 4 Endogenous erythropoietin (EPO, ln U/L) and a relative hazard of all-cause death using cubic splines during the 12-month follow-up in patients with heart failure and anaemia.

had measured EPO and were included in aforementioned analyses. Second, follow-up was available for 283 patients and therefore 1-year survival analyses are limited to this number of subjects. Moreover, we do not have precise data on patients' treatment (including intravenous iron, blood transfusions or ESA) before the inclusion in the study (months preceding enrolment) as well as during the follow-up period. Another limitation is that laboratory tests analysed in this study were performed only at baseline and there is no follow-up available with regard to either iron status or the effectiveness of erythropoiesis in examined subjects. Finally, we analysed CRP as the only biomarker of inflammation and it would be worth investigating this aspect more comprehensively by including a wider portfolio of pro-inflammatory cytokines and their receptors.[37]

Conclusions

The majority of anaemic HF patients have abnormal EPO level, and patients with endogenous EPO deficiency vs. resistance have different clinical and laboratory characteristics. In this patient cohort, depleted iron stores contribute to the resistance to endogenous EPO independently of inflammation. Although we have demonstrated the trend towards increased 12-month mortality in subjects with elevated EPO, the prognostic significance of endogenous EPO resistance in anaemic HF patients requires further research.

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