Adequacy of endogenous erythropoietin levels and mortality in anaemic heart failure patients

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Aims
We examined the adequacy of endogenous erythropoietin (EPO) levels for the degree of anaemia in patients with chronic heart failure (CHF) and its relation to prognosis.

Methods and results
We studied 74 anaemic CHF patients from a cohort of 240 patients. The adequacy of endogenous EPO levels was assessed by derived observed/predicted (O/P) ratio. A ratio value <0.92 indicates EPO levels lower than expected, whereas a value >1.09 indicates EPO levels higher than expected. The primary endpoint was mortality. During a median follow up of 4.9 years, 35 of the 74 (47.3%) anaemic patients died. EPO levels lower than expected were observed in 29 patients (39%), whereas EPO levels higher than expected were present in 22 anaemic patients (29%). The Kaplan–Meier analysis revealed that anaemic patients with EPO levels higher than expected had a significantly higher mortality rate compared to patients with EPO levels as expected or EPO levels lower than expected (log-rank: P = 0.024). A higher O/P ratio was an independent predictor of increased mortality risk adjusted for variables including age, sex, haemoglobin, NT-proBNP, and renal function; hazard ratio (HR): 1.020 95%CI (1.004–1.036), P = 0.012.

Conclusion
EPO levels higher than expected, suggesting resistance to the hormone, are common in CHF patients and are associated with a higher mortality.

Keywords
Heart failure • Anaemia • Erythropoietin • Prognosis

Introduction
Anaemia is commonly observed in patients with chronic heart failure (CHF). The prevalence of anaemia depends both on the severity of heart failure and the diagnostic criteria, but may be as high as 55%. In addition, among patients with CHF, anaemia is associated with an impaired prognosis and more severe symptoms. However, while it is both common and relevant from a prognostic perspective, anaemia in such patients may be due to a wide range of aetiologies. One aetiology yet studied among anaemic patients with CHF is the phenomenon of bone marrow resistance to endogenous effects of erythropoietin (EPO), an important cause of anaemia in patients with chronic diseases other than CHF.

Previously, we and others have shown that elevated endogenous EPO levels are observed in CHF patients and are associated with an impaired survival, independent of haemoglobin levels. However, among these subjects, for a given haemoglobin concentration, we observed large variations in endogenous EPO levels with a subgroup of patients demonstrating high endogenous EPO levels and only marginally low haemoglobin levels. Considering the possible mechanisms to explain this observation, those with higher than expected EPO levels, might theoretically be characterized as those resistant to endogenous EPO, while others with...
either low or more appropriate levels of EPO relative to their anaemia could be characterized as having normal or elevated sensitivity to endogenous EPO in the bone marrow; given this possible scenario.

We, therefore, aimed to study the relation between endogenous EPO levels and haemoglobin levels in anaemic CHF patients and related this to outcome. Our hypothesis was that EPO resistance would be common among patients with CHF and in those CHF patients with a phenotype of EPO resistance we would observe a higher rate or mortality.

Methods

Patients

All patients in the present study were recruited into a prospective multicentre CHF study evaluating the influence of protocolized intervention by clinician and cardiovascular nurse, as previously described. Referral of patients to our clinic was by general practitioners, cardiologists of other local hospitals, or other units of the hospital. Patients, either hospitalized or visiting the outpatient clinic, with New York Heart Association (NYHA) III or IV were eligible for the study. In all patients, CHF was diagnosed on the basis of standard criteria, and the presence of left ventricular enlargement or systolic functional impairment by radionuclide ventriculography or echocardiography, according to the European Society of Cardiology guidelines. Exclusion criteria were: dementia, psychiatric illness, discharge, or stay in nursing home, diseases other than CHF with a life expectancy less than 1 year, ongoing or planned hospitalization and kidney function replacement. All subjects gave informed consent for the protocol, which was approved by the local Medical Ethics Committee. The total cohort consisted of 240 CHF patients, of which 77 were anaemic (32%). In the present analysis, we included 74 anaemic patients with complete datasets of plasma EPO, NT-proBNP, renal function, biometric measurements, and medication use.

Study endpoints

Patients were recruited between March 2000 and April 2003 and followed until 15 September 2006. Mortality was assessed by review of the medical record or contacting the general practitioner or the heart failure clinic. There was no loss-to follow-up. The primary end-point was all-cause mortality.

Renal function, haemoglobin level, and ferritin

The glomerular filtration rate (GFR) is a standard indicator of renal function. Under steady-state conditions, GFR is estimated from serum creatinine using a formula that accounts for the influence of age on creatinine production [simplified modification of diet in renal disease (sMDRD)]. GFR: Male: 186.3 × (serum creatinine)−1.154 × (age)−0.203; black male: sMDRD × 1.212; female: sMDRD × 0.742; black female: sMDRD × 1.212 × 0.742, which has been validated in CHF patients. Anaemia was defined as a haemoglobin level <13.0 g/dL for men and postmenopausal women and <12.0 g/dL for premenopausal women, as previously described. Ferritin levels were measured in stored plasma. According to Thomas and Thomas, the cut-off values for ferritin deficiency were dependent on the acute-phase response. Ferritin deficiency was defined as <20.8 μg/L in patients with high sensitive (hs)-C-reactive protein levels ≤5 mg/L, and <61.7 μg/L in patients with hs-C-reactive protein levels >5 mg/L.

Erythropoietin

Plasma EPO levels were measured using the IMMULITE® EPO assay (DPC, Los Angeles, CA, USA), which has been described before. The adequacy of endogenous EPO levels is assessed by the observed/predicted (O/P) ratio, as previously described. A value below 1 suggests that endogenous EPO production is lower than expected, whereas a value above 1 suggests that endogenous EPO production is higher than expected. To define EPO levels appropriate or inappropriate for a given degree of anaemia, we obtained a reference sample of anaemic patients without cardiac and renal disease and hs-C-reactive protein levels ≤5 mg/L. In total, 143 patients were screened for the reference sample. In ten patients, EPO levels could not be measured due to insufficient blood collection. Of the remaining 133 patients, 23 were considered anaemic. Two patients were excluded due to renal failure and one was excluded due to elevated hs-C-reactive protein levels. Eventually, the 20 remaining patients were included in the reference sample. By using the haemoglobin and EPO levels, a regression equation was constructed: log(EPO) = −4.640 − (0.274 × Hb), which is well comparable to a previous study in CHF patients. This equation was used to predict the EPO levels based on the haemoglobin level: predicted log(EPO) levels. The actual observed log(EPO) levels in our CHF patients was divided by the predicted log(EPO) values to create an O/P ratio. The mean O/P ratio for the reference subjects was 1.003 [95% confidence interval (CI) 0.916–1.087]. Lower than expected was defined as an O/P < 0.916, while EPO level higher than expected was defined as an O/P > 1.087, based on the 95% CI of the reference population.

Statistics

Data are given as mean ± SD when normally distributed, as median and interquartile range (IQR) when skewed, and as frequencies for categorical variables. We compared differences between groups with the Mann–Whitney U test, Kruskall–Wallis test, student t-test, ANOVA with Bonferroni post hoc testing, and Pearson χ² test when appropriate. Kaplan–Meier method was used to study the influence of the O/P ratio of EPO levels on survival, with log-rank testing. Furthermore, we used the Cox proportional hazard analysis to assess the association between O/P ratio of EPO levels and mortality. Regarding the limited sample size, in the multivariable model, we corrected for five risk factors known to be important predictors of mortality in CHF patients including: age, sex, nt-proBNP, renal function, and haemoglobin. Hazard ratios (HRs) with 95% CIs demonstrated the risk of death. The validity of the proportional hazard assumption was checked using the Schoenfeld residuals. The proportional hazard assumption was tested for each covariate in the multivariable analysis. Plots of Schoenfeld residuals against the time were made. No violations were found. Spearman correlation coefficients were calculated to determine the correlations between O/P ratio of EPO levels and hs-C-reactive protein. All reported probability values were two-tailed, and a P-value <0.05 was considered statistically significant. For all statistical analysis, SPSS version 13.0 was used.

Results

The average age of the anaemic patients was 73 ± 8 years, 68% were male, and the average left ventricular ejection fraction was 30 ± 8%. The majority of patients were in NYHA class III (92%). Of the 74 anaemic patients, 35 patients (47.3%) died after 26 days to 5.9 years. Median follow-up period of the 39 survivors was 4.9 years (IQR 4.3–5.9).
Adequacy of erythropoietin production

Median endogenous EPO levels were 23.4 U/L (IQR 14.0–45.1); (range: 7.70–276). The average haemoglobin level in the anaemic cohort was 11.7 ± 1.0 g/dL. The majority of the patients included in the current study had an ischaemic aetiology (76%). We only found a non-significant difference in EPO levels between patients with ischaemic CHF and non-ischaemic CHF; 25.7 U/L (15.2–52.5) and 17.4 U/L (11.2–36.9), respectively. The O/P ratios were divided in three groups, as described in the methods section: lower than predicted EPO levels (39%; n = 29), higher than predicted EPO levels (29%, n = 22), and EPO levels as predicted (31%, n = 23). The baseline characteristics of the anaemic patients are depicted in Table 1. The mean O/P ratio was 1.01 ± 0.26. As expected, cGFR was significantly lower in patients with EPO levels lower than expected (P = 0.004). Haemoglobin levels were slightly higher in patients with unexpectedly high EPO levels (P = 0.01). Also C-reactive protein levels were higher in patients with EPO levels higher than expected, although this did not reach statistical significance. We observed a positive correlation between C-reactive protein and O/P ratio (r = 0.277, P = 0.017). Ferritin deficiency was mostly observed in patients with EPO levels as expected (P = 0.04).

Adequacy of erythropoietin production and survival

Higher than expected EPO levels were associated with a higher mortality rate compared to patients with EPO levels as expected or those with lower than expected EPO levels. Kaplan–Meier survival curves for the three groups showed an increased mortality over time in patients with higher than expected EPO levels (log rank: P = 0.024; Figure 1). The multivariate regression analysis revealed that a higher O/P ratio, suggesting EPO resistance, was an independent predictor of increased mortality risk in Cox-regression analyses adjusted for variables including age, sex, haemoglobin, NT-proBNP, and cGFR; HR: 1.020 95% CI (1.004–1.036), P = 0.012 (Table 2).

Discussion

The main and novel finding of the present study is that higher than expected EPO levels in anaemic heart failure patients are frequently present and are strongly associated with a higher mortality compared to anaemic CHF patients with (lower than) expected EPO levels. Mechanistically, anaemic CHF patients with higher than expected EPO levels are obviously capable of producing endogenous EPO, but their bone marrow might be resistant to the hormone. These patients should be distinguished from anaemic CHF patients with lower than expected EPO levels, in which the primary problem probably reflects renal dysfunction, but who have a normal or even increased sensitivity to EPO. This is supported by our observation that renal dysfunction was more frequent amongst the group of patients with lower than expected EPO levels.

Few studies have been performed on the role of endogenous EPO levels in CHF. It has been observed that endogenous EPO levels in CHF patients are generally elevated; proportional to the severity of symptoms.18,19 In a relatively small study, we previously showed that the plasma EPO and haemoglobin levels were independent predictors of survival.6 These findings were recently confirmed by others in a slightly larger CHF population.9 We additionally demonstrated only a mild inverse correlation between EPO and haemoglobin levels in CHF patients, whereas the control group showed a clear significant inverse correlation, as expected. These findings already suggested large variations in EPO levels for given haemoglobin levels in CHF patients. In the present study, we focused on O/P ratios of EPO in CHF patients to further explore the adequacy of endogenous EPO levels in anaemic CHF patients.

With respect to adequacy of the endogenous EPO level relative to the severity of anaemia, we suggest that proposed O/P ratio allows for a better mechanistic understanding of the cause of anaemia. Indeed, while the observation that the effects of EPO may be affected in patients with CHF has been made, the explanation for this finding or the prognostic relationships between this finding remained less clear. Higher than expected EPO levels have been observed as a primary response in patients after intensive chemotherapy, although mechanistically it remains unclear why this may be and how it might be related to our subjects with CHF.20 The association between EPO levels and inflammation in those with possible EPO resistance has been described previously.6,21,22 In addition, in vitro data have shown that greater amounts of EPO are needed to restore proliferation of bone marrow-derived cells when inflammatory cytokines are present.23 and indeed in our analysis, higher than expected EPO levels correlated with C-reactive protein concentrations in subjects so afflicted. The effects of inflammation in CHF are numerous,24,25 although to our knowledge a unifying theory for inflammation, anaemia, and CHF has not been previously proposed. Another theory may be that a circulating inhibitor to haematopoiesis may be present in those with higher than expected EPO concentrations. Previous studies from our group showed that serum of CHF patients inhibits the proliferation of bone marrow derived erythropoietic cells from healthy volunteers, indicating that serum factors induce insensitivity to endogenous EPO.26 We established that serum levels of Ac-SDKP, a strong haematopoiesis inhibitor, were significantly higher in these anaemic CHF patients. Since ACE is the principle enzyme metabolizing Ac-SDKP, the ramification for those anaemic patients treated with ACE-inhibitors is clear.

We acknowledge that the mechanisms of anaemia in CHF are many. Indeed, lower than expected EPO values were also frequently seen in our cohort. Chronic kidney disease (CKD) is likely to be a significant contributory factor in the anaemia observed in CHF patients. In many patients with end-stage renal disease, anaemia is a common feature. In turn, CHF can cause CKD due to decreased cardiac output and relative renal vasoconstriction, leading to chronic renal ischaemia, CKD, and ultimately anaemia. The links between CKD, CHF, and anaemia have been called the Cardio-Renal-Anaemia syndrome.27 The current study supports the concept that anaemia develops due to the inability of the kidney to produce sufficient levels of EPO to stimulate the bone marrow adequately. Lastly, adequate EPO levels for the degree of anaemia are mostly observed in patients with low ferritin
levels.\textsuperscript{21} Our study confirms these findings since almost 50% of the anaemic patients with adequate EPO levels had low ferritin values.

Several limitations of the present study have to be acknowledged. The analysis of the anaemic subgroup was based on a relatively small sample size, although the mortality rate was almost 50%. We did not measure cytokine levels nor Ac-SDKP levels which may play an important role in the resistance of the bone marrow for endogenous EPO in CHF. Because of these limitations, we regard our study mainly as a hypothesis-generating study. Nevertheless, our findings suggest that resistance to endogenous EPO is common in anaemic CHF patients and relates to an

<table>
<thead>
<tr>
<th>Variable</th>
<th>EPO levels lower than expected (n = 29)</th>
<th>EPO levels as expected (n = 23)</th>
<th>EPO levels higher than expected (n = 22)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>72.7 ± 8.1</td>
<td>72.6 ± 9.4</td>
<td>72.3 ± 6.1</td>
<td>0.98</td>
</tr>
<tr>
<td>Sex (n/% male)</td>
<td>16 (55.2)</td>
<td>15 (65.2)</td>
<td>19 (86.4)</td>
<td>0.06</td>
</tr>
<tr>
<td>Ischaemic aetiology (n/%)</td>
<td>21 (72.4)</td>
<td>18 (78.3)</td>
<td>17 (77.3)</td>
<td>0.31</td>
</tr>
<tr>
<td>NYHA class III (n/%)</td>
<td>26 (89.7)</td>
<td>21 (91.3)</td>
<td>21 (95.5)</td>
<td>0.75</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122 ± 29.2</td>
<td>121 ± 19.0</td>
<td>122 ± 19.7</td>
<td>0.98</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>32 ± 7.8</td>
<td>30 ± 9.0</td>
<td>28 ± 9.0</td>
<td>0.36</td>
</tr>
<tr>
<td>NT-proBNP (pmol/L)</td>
<td>265 (109–862)</td>
<td>367 (200–614)</td>
<td>533 (435–904)</td>
<td>0.48</td>
</tr>
<tr>
<td>O/P (%)</td>
<td>0.77 ± 0.08</td>
<td>1.00 ± 0.05</td>
<td>1.32 ± 0.19</td>
<td>–</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.4 ± 1.0</td>
<td>11.6 ± 0.9</td>
<td>12.2 ± 0.7\textsuperscript{6}</td>
<td>0.007</td>
</tr>
<tr>
<td>Mean corpuscular volume (fl)</td>
<td>88.9 ± 5.2</td>
<td>90.1 ± 7.0</td>
<td>90.4 ± 7.4</td>
<td>0.69</td>
</tr>
<tr>
<td>Erythropoietin (U/L)</td>
<td>13.0 (10.7–16.6)</td>
<td>24.1 (16.6–47.7)</td>
<td>43.7 (26.2–77.4)\textsuperscript{6}</td>
<td>0.001</td>
</tr>
<tr>
<td>hs-C-reactive protein (mg/L)</td>
<td>13 (4.5–27)</td>
<td>6 (2–22)</td>
<td>14 (4–37.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>cGFR (mL/min/1.73 m\textsuperscript{2})</td>
<td>41.4 ± 10.8\textsuperscript{1}</td>
<td>54.1 ± 16.5</td>
<td>46.3 ± 16.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Ferritin deficiency (%)</td>
<td>4 (13.8)\textsuperscript{8}</td>
<td>10 (43.5)</td>
<td>5 (22.7)\textsuperscript{8}</td>
<td>0.04</td>
</tr>
<tr>
<td>Medication (n/% use)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-inhibitor</td>
<td>26 (89.7)</td>
<td>20 (87.0)</td>
<td>18 (81.0)</td>
<td>0.68</td>
</tr>
<tr>
<td>Angiotensin receptor blocker</td>
<td>3 (10.3)</td>
<td>5 (21.7)</td>
<td>2 (9.5)</td>
<td>0.40</td>
</tr>
<tr>
<td>Diuretics</td>
<td>28 (96.6)</td>
<td>23 (100)</td>
<td>22 (100)</td>
<td>0.47</td>
</tr>
<tr>
<td>Digoxin</td>
<td>8 (27.6)</td>
<td>5 (21.7)</td>
<td>8 (38.1)</td>
<td>0.49</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>20 (69.0)</td>
<td>17 (73.9)</td>
<td>18 (81.0)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

LVEF, left ventricular ejection fraction; O/P, observed/predicted; cGFR, calculated glomerular filtration rate.
\*P < 0.05 vs. adequate EPO response.
\textsuperscript{1}P < 0.01 vs. adequate EPO response.
\textsuperscript{2}P < 0.05 vs. inadequate low EPO response.
\textsuperscript{3}P < 0.01 vs. inadequate low EPO response.

Figure 1 Kaplan–Meier survival curve for all cause mortality for the three different groups. EPO levels lower than expected (n = 29), EPO levels as expected (n = 23), and EPO levels higher than expected (n = 22).
impaired prognosis. This may help to understand the pathophysiology of anaemia in CHF and the relation between elevated endogenous EPO levels and mortality. Furthermore, it would be interesting to evaluate whether (anaemic) CHF patients with EPO resistance are also resistant to exogenous EPO, and whether such patients have comparable or worse prognosis to those without such resistance. Currently, a large randomized placebo-controlled trial is being conducted to investigate the role of exogenous EPO in anaemic CHF patients.28

Conflict of interest: none declared.

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References
21. Theurl I, Mattie V, Seifert M, Mariani M, Marth C, Weiss G. Dysregulated monocyte iron homeostasis and erythropoietin formation

Table 2 Multivariable predictors of all-cause mortality in the anaemic cohort (n = 74)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Multivariable</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>CI</td>
<td>P</td>
</tr>
<tr>
<td>O/P ratio (%)</td>
<td>1.029</td>
<td>1.004–1.036</td>
<td>0.012</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>2.541</td>
<td>1.089–5.805</td>
<td>0.031</td>
</tr>
<tr>
<td>cGFR (mL/min/1.73 m²)</td>
<td>0.958</td>
<td>0.931–0.985</td>
<td>0.005</td>
</tr>
<tr>
<td>Age (10 years)</td>
<td>1.335</td>
<td>0.817–2.182</td>
<td>0.249</td>
</tr>
<tr>
<td>ntrproBNP (10 pmol/L)</td>
<td>1.001</td>
<td>0.996–1.005</td>
<td>0.814</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>0.748</td>
<td>0.367–1.521</td>
<td>0.422</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, 95% confidence intervals; O/P, observed predicted ratio; cGFR, calculated glomerular filtration rate.


**CLINICAL VIGNETTE**

Thoracic mycotic pseudoaneurysm from *Candida albicans* infection

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A 33-year-old man was admitted to the emergency department with fever, chills since 3 weeks and an elevated C-reactive protein. Two years ago, he had undergone an aortic valve reconstruction and mitral valve replacement owing to endocarditis. Three months prior to admission, he suffered from sternal osteomyelitis caused by infected cerclages with a smear positive for *Candida albicans*. Both artificial valves did not present signs of endocarditis. Blood cultures were tested negative. Serology was tested positive for *C. albicans*. CT scans demonstrated a localized mycotic pseudoaneurysm of the ascending aorta and retrosternal hemorrhage (Panel A). Histology of the felt-like aortic tissue demonstrated amorphous material with an inflammatory edge in the H&E staining (Panel B). The Grocott staining, specific for fungal structures, was strongly positive and demonstrated multiple black layers of fungus (Panels C and D). The defect was closed with a patch and the patient recovered completely after long-term antimycotic treatment.

Panel A: The Multidetector-Row CT scan (64-slice CT) of the chest demonstrates a localized pseudoaneurysm of the ascending aorta with retrosternal hemorrhage.

Panel B: Micrograph of the surgical specimen from the aortic wall showing amorphous material with an inflammatory edge (Hematoxylin & eosin stain, original magnification × 200).

Panel C: Micrograph demonstrates multiple black layers of fungus (Grocott stain, original magnification × 100).

Panel D: Higher magnification detected round fungus spores with longish hyphae indicating Candida sp. (Grocott stain, original magnification × 400).

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