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Anaemia in chronic heart failure is not only related to impaired renal perfusion and blunted erythropoietin production, but to fluid retention as well

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Extracellular volume

Aims Anaemia is prevalent in the chronic heart failure (CHF) population, but its cause is often unknown. The present study aims to investigate the relation between anaemia, renal perfusion, erythropoietin production, and fluid retention in CHF patients.
Methods and results We studied 97 patients with CHF, of which 15 had anaemia (Hb $<$13.0 g/dL in men and Hb $<$12.0 g/dL in women), without haematinic deficiencies. Glomerular filtration rate (GFR) and extracellular volume (ECV) were measured as the clearance and the distribution volume of constantly infused ^{125}I-iothalamate, respectively. Effective renal plasma flow (ERPF) was determined as the clearance of ^{131}I-hippuran. Anaemic CHF patients displayed significantly reduced GFR ($P = 0.002$), ERPF ($P = 0.005$) and EPO production ($P = 0.001$), and an elevated ECV ($P = 0.015$). Multivariable analysis demonstrated that lower GFR ($P = 0.003$), lower ERPF ($P = 0.004$), lower EPO production ($P = 0.006$), and a higher ECV ($P = 0.001$) were significant independent predictors of lower haemoglobin levels.
Conclusion Anaemia in CHF is not only independently associated with impaired renal perfusion and blunted EPO production, but to fluid retention as well.

Introduction

Anaemia is present in a substantial part of the chronic heart failure (CHF) population, ranging from 15–55%, depending on the definition of anaemia and severity of disease.[1] Anaemia is independently associated with increased morbidity and impaired prognosis, although the cause of anaemia is frequently unknown.[1–5] CHF is associated with elevated levels of erythropoietin (EPO), suggesting impaired erythropoietic activity in the bone marrow.[1,6,7] Recently, we demonstrated that anaemia in CHF could partly be explained by increased serum levels of AcSDKP, a negative regulator of haematopoietic stem cell proliferation.[8] In addition, we hypothesize that CHF will compromise renal perfusion resulting in impaired EPO production, thereby causing anaemia. Finally, it has been suggested that anaemia in CHF may be partly explained by fluid retention and consequent haemodilution.[9] However, the relative contribution of renal perfusion, EPO production, and fluid retention to the presence of anaemia in CHF has so far not been well described. We therefore evaluated the relation between anaemia, effective renal plasma flow (ERPF), EPO production, and extracellular volume (ECV) in CHF patients.

Methods

Patient population

Clinically stable CHF patients on outpatient follow-up at our department were asked to participate, as described in detail previously.[10] Approximately 121 patients were asked to participate. In total, 110 patient were included into the original analysis and finished the study. Owing to missing haemoglobin levels, 13 patients were excluded form analysis, leaving 97 subjects for analysis. Briefly, inclusion criteria were age $>$18 years and left ventricular ejection fraction (LVEF) $<$45%. All patients used renin–angiotensin system blockers, and medication had remained stable for at least 1 month. Exclusion criteria included stroke, myocardial infarction, or cardiac revascularization procedures within the last 3 months or scheduled for these procedures, unstable angina, primary renal disease, prior organ transplant, or chronic use of renal function compromising medication.

Cardio-renal haemodynamic parameters

LVEF was determined by nuclear ventriculography or echocardiography using Simpsons rule. Mean arterial pressure (MAP) was

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calculated from systolic and diastolic blood pressure measurements obtained immediately before ^{125}I-iotholamate and ^{131}I-hippuran clearance measurements from 10 consecutive measurements in supine position using an automated system. N-terminal proBNP (NT-proBNP) was determined by electrochemiluminescence immunoassay on the Roche Elecsys (Roche diagnostics, Netherlands). Glomerular filtration rate (GFR) and ERPF were measured by constant infusion of the radiolabelled tracers ^{125}I-iothalamate and ^{131}I-hippuran.[11] Briefly, after drawing a blank blood sample, a priming solution containing 0.4 mL/kg body weight of the infusion solution (0.04 MBq of ^{125}I-iothalamate and 0.03 MBq of ^{131}I-hippuran) plus an extra amount of 0.6 MBq of ^{125}I-iothalamate was given at 8 a.m., followed by infusion at 12 mL/h, adapted to 9 mL/h in subjects with renal function impairment as estimated from previously obtained serum creatinine values. This ensures steady-state plasma levels of ^{131}I-hippuran and ^{125}I-iothalamate after a run-in period of 2 h, as verified by hourly blood samples. Subsequently, clearances of ^{125}I-iothalamate and ^{131}I-hippuran and the distribution volume of ^{125}I-iothalamate were measured during steady state. The GFR and ERPF were calculated as $(U\cdot V)/P_{(\text{iothalamate})}$ and $(I\cdot V)/P_{(\text{hippuran})}$, respectively, and $(U\cdot V)/P_{(\text{iothalamate})}$ was corrected for voiding errors by the ratio of the urinary to plasma clearance of ^{131}I-hippuran. $U\cdot V$ represents the urinary excretion of the tracer and $I\cdot V$ the infusion rate of the tracer; P represents the values in plasma calculated from the samples bracketing each clearance period. The body surface area (BSA) was calculated as $0.007184\cdot\text{weight}^{0.425}\cdot\text{length}^{0.725}$, and GFR and ERPF were expressed per 1.73 m^2 of BSA. Renal blood flow (RBF) was calculated as ERPF/1-haematocrit. The filtration fraction (FF) was calculated as the ratio of GFR and ERPF and expressed as percentage. ECV was estimated from the distribution volume of ^{125}I-iotholamate[12,13] and calculated as $[(I\cdot V + B\cdot V) - U\cdot V]/P_{\text{iothalamate}}$ during steady state. $B\cdot V$ represents the bolus infusion of the tracer. GFR and ERPF were expressed per 1.73 m^2 of BSA, and ECV was expressed as L/kg body weight.

Haemoglobin levels, haematinic parameters, and EPO levels

Haemoglobin, iron, ferritin, transferrin, and vitamin B11 and B12 levels were determined at the local laboratory facilities. EPO levels were determined by IMMULITE EPO assay (DPC, Los Angeles, CA, USA). To define the relation between EPO levels and a given Hb, we included 15 reference subjects referred to our department with complaints of chest pain or palpitations. The reference subjects had a mean age of 50 $\pm$ 4.5 and had normal LVEF (LVEF $>$ 60%), normal renal function, no signs of inflammation, or symptoms of CHF.[14] An exponential regression equation of serum EPO vs. Hb (mmol/L) was calculated, resulting in the following equation $\log \text{EPO} = 3.015 - (0.130 * \text{Hb})$. Predicted log EPO and observed/predicted log EPO ratio (log serum EPO/predicted log EPO) were calculated with this equation. Mean O/P ratio in reference subjects was 0.90 $\pm$ 0.029 (95% CI 0.64–1.12). Total iron binding capacity (TIBC) was calculated by multiplying serum transferrin $\times$ 20. Transferrin saturation (FeSat) was calculated as (serum iron/TIBC) * 100%. Iron deficiency was defined as ferritin levels $<$30 μg/L or FeSat $<$15%. According to local laboratory reference ranges, deficiency in vitamin B11 and B12 was defined as levels $<$142 pmol/L and 50 pg/mL, respectively.

High-sensitivity CRP was determined by nepholometry. The threshold for detection was 0.156 mg/L; when CRP levels were below the detection limit, they were assigned the value 0.156 mg/L for statistical purposes.

Statistics

Data are given as mean $\pm$ standard deviation when normally distributed, as median and interquartile range when skewed distributed, and as frequencies and percentages for categorical variables. Differences between groups were compared with Student's t-test, Mann–Whitney U test, χ^2 of Fisher's exact test where appropriate. A P-value $<$0.05 was considered statistically significant, and all reported probability values are two-sided. Correlation between Hb, EPO or O/P ratio, and various other variables was performed using Pearson's correlation coefficients. Non-normally distributed continuous variables were log-transformed. The variables age, sex, pharmacological treatments, New York Heart Association (NYHA) functional class, LVEF, ERPF, GFR, FF, ECV, NT-proBNP, CRP, and MAP were assessed for univariate linear association with Hb or log EPO. Variables that showed a significant ($P < 0.15$) univariate association were included stepwise in a multivariable linear regression model on the basis of on the strength of the univariate association. All the variables described earlier were added to the final model simultaneously to assure that addition of these variables did not significantly increase the predictive accuracy of the model. The final model was assessed for first line interaction.

Results

Patient characteristics

Seventy six percent of subjects were male and age ranged from 27 to 81 years. NYHA functional classes I, II, III, and IV comprised 14, 44, 31, and 10% of patients, respectively.

Difference in characteristics between anaemic and non-anaemic CHF patients

In the total population, 19 patients (20%) were anaemic according to the WHO criteria (Hb $<$13.0 g/dL in men and Hb $<$12.0 g/dL in women). Iron deficiency was present in four out of 19 anaemic (21%) and three out of 78 non-anaemic (4%) CHF patients. Other haematinic deficiencies were not observed. The iron-deficient patients were excluded from further analysis, leaving 75 non-anaemic subjects and 15 subjects with unexplained anaemia. Differences in characteristics between anaemic and non-anaemic subjects are summarized in *Table 1*. Anaemic subjects were significantly older and in a higher NYHA class. Although LVEF was comparable, compared with non-anaemic patients, anaemic patients showed more severe haemodynamic impairment, reflected by reduced MAP (86.8 $\pm$ 13 vs. 76.8 $\pm$ 14 mmHg; $P = 0.007$), ERPF (286 $\pm$ 83 vs. 219 $\pm$ 74 mL/min/1.73 m^2; $P = 0.005$) and RBF (502 $\pm$ 150 vs. 348 $\pm$ 121 mL/min/1.73 m^2; $P < 0.001$), and elevated NT-proBNP levels [10 (260–1355) vs. 1004 (720–1904) pg/mL; $P = 0.029$]. Anaemic CHF patients were more often using diuretics (65 vs. 87%; $P = 0.045$) and despite this displayed significantly elevated ECV (0.25 $\pm$ 0.5 vs. 0.29 $\pm$ 0.4 L/kg; $P = 0.015$), implicating fluid overload. The fluid retention was subclinical, as anaemic patients did not display oedema, nocturia, or dyspnoea more frequently (data not shown). Plasma sodium levels and fractional sodium excretion were similar, implicating that the elevated ECV was not caused by excess sodium intake. Although creatinine levels were comparable between groups, urea levels were elevated [19 (16–22) vs. 35 (23–40) mg/dL; $P = 0.007$] and GFR (79 $\pm$ 25 vs. 56 $\pm$ 28 mL/min/1.73 m^2; $P = 0.002$) was significantly reduced in anaemic patients. Moreover, anaemic patients had a higher incidence of moderate renal failure (GFR $<$60 mL/min/1.73 m^2) and a trend towards more severe renal failure (GFR $<$ 30 mL/min/1.73 m^2) (21 vs. 60%, $P < 0.005$ and 5 vs. 20%, $P = 0.088$, respectively).

Table 1 Characteristics of anaemic and non-anaemic CHF patients

	Non-anaemic ($n = 75$)	Anaemic ($n = 15$)	*P*-value
Age (years)	56.3 ± 12	65.6 ± 9	0.004*
Sex (*n*, % male)	58 (85)	10 (71)	0.510
NYHA class	2.3 ± 0.8	2.8 ± 0.8	0.036*
BMI (kg/m^2)	27.8 ± 3.9	26.1 ± 3.0	0.140
Ischaemic aetiology (*n*, %)	38 (50)	7 (47)	1
Cardiorenal haemodynamic parameters			
Heart rate	65 ± 2	64 ± 3	0.834
MAP (mmHg)	86.8 ± 1.5	76.8 ± 3.6	0.007*
LVEF (%)	28 ± 10	26 ± 6	0.525
NT-proBNP (pg/mL)	510 (260–1355)	1004 (720–1904)	0.029*
Creatinine (mg/dL)	1.2 (1–2)	1.2 (1.1–1.7)	0.150
Urea (mg/dL)	19 (16–22)	35 (23–40)	<0.001*
Plasma sodium (mEq/L)	137 ± 3	136 ± 3	0.589
GFR (mL/min /1.73 m^2)	79 ± 25	56 ± 28	0.002*
ERPF (mL/min/1.73 m^2)	286 ± 83	219 ± 74	0.005*
RBF (mL/min/1.73 m^2)	502 ± 150	348 ± 121	<0.001*
FF (%)	28 (26–30)	26 (20–29)	0.370
FENa (%)	0.88 ± 0.33	0.97 ± 0.52	0.370
ECV/body weight (L/kg)	0.25 ± 0.5	0.29 ± 0.4	0.015*
Mild RF (GFR < 60)	16 (21%)	9 (60%)	0.004*
Severe RF (GFR < 30)	4 (5%)	3 (20%)	0.088
Erythropoietic and inflammatory parameters			
Hb (mg/dL)	15 ± 0.7	12.7 ± 0.4	<0.001*
Serum EPO (*U/L*)	15.7 (11–21)	18.5 (12–31)	0.357
O/P ratio	1.15 ± 0.20	0.93 ± 0.18	0.001*
CRP (mg/L)	2.15 (0.95–4.06)	2.38 (0.77–5.66)	0.733
Medication			
ACE-inhibitors, *n* (%)	66 (88)	12 (80)	1
ARB, *n* (%)	9 (12)	3 (20)	0.414
Beta-blockers, *n* (%)	62 (83)	13 (87)	1
Diuretic, *n* (%)	49 (65)	13 (87)	0.045*
Aldosterone ant. (%)	51 (88)	7 (47)	0.144

All continuous variable are presented as mean ± SE if normally distributed and as median value with 25th–75th percentile when skewed distributed. BMI, body mass index; FENa, fractional Na excretion; Hb, haemoglobin; RF, renal failure; *O/P* ratio, ratio between observed and predicted log EPO; CRP, C-reactive protein; ARB, angiotensin receptor blocker; aldosteron ant, aldosteron antagonists.

*$P < 0.05$.

By definition, Hb level was significantly lower in anaemic subjects. However, EPO levels were comparable between anaemic and non-anaemic CHF patients. Additionally observed/predicted (*O/P*) ratio was significantly reduced in anaemic subjects (1.15 ± 0.2 vs. 0.93 ± 0.2, $P = 0.001$), indicating blunted EPO production. *O/P* ratio was significantly higher in non-anaemic CHF patients compared with reference subjects ($P = 0.026$), whereas *O/P* ratio in anaemic CHF patients and controls were comparable. Thus, EPO production is elevated in both anaemic and non-anaemic CHF patients. But based on their Hb, it should have been higher in anaemic CHF patients. It therefore seems that the compensatory rise in response to anaemia is impaired.

Hb levels and EPO production in the CHF population

As previously described, CHF patients displayed a relatively moderate negative correlation between EPO and Hb levels ($R = -0.281$, $P = 0.007$). A moderate significant correlation was also observed between EPO levels and both CRP ($R = 0.281$, $P = 0.007$) and NYHA class ($R = 0.210$, $P = 0.05$), and a trend with NT-proBNP ($R = 0.193$, $P = 0.07$). No significant correlation was observed between EPO levels and other markers for cardiorenal haemodynamic status, or renal function parameters. *O/P* ratio correlated with Hb ($R = 0.397$, $P = 0.001$), GFR ($R = 0.237$, $P = 0.024$), and FF ($R = 0.285$, $P = 0.007$).

Predictors of Hb and serum EPO

Univariate and multivariable linear associations between Hb and EPO levels are displayed in *Tables 2* and *3*, respectively. Sex, lower EPO (*Figure 1A*), lower GFR (*Figure 1B*), lower ERPF (*Figure 1C*), and higher ECV (*Figure 1D*) were independently associated with lower Hb levels, accounting for 31–44% of the variance in Hb levels. The variables CRP, NYHA class, NT-proBNP, and Hb showed significant univariate association with plasma EPO. However, higher CRP and lower Hb levels were the only independent predictors of higher serum EPO levels. Inclusion of the full list of possible predictive variables did not result in a significant increase in the adjusted R^2, slope, or partial correlation coefficient of the variables in our model.

Table 2 Univariate and multivariable predictors of Hb levels

Haemoglobin								
Variable	Univariate			Multivariable				
	B	SE	*P*-value	B	SE	β	Part. cor.	*P*-value
Sex	−1.41	0.30	<0.001	−1.189	0.258	−0.394	−0.449	<0.001
Age	−0.022	0.012	0.077					
GFR	0.02	0.005	<0.001	0.037	0.009	0.744	0.414	<0.001
ERPF	−0.004	0.002	0.013	−0.008	0.003	−0.534	−0.310	0.004
NYHA	−0.04	0.17	0.013					
EPO	−1.55	0.57	0.007	−1.422	0.430	−0.266	−0.399	0.001
ECV	−6.36	2.95	0.034	−7.120	2.215	−0.259	−0.330	0.004
MAP	−0.03	0.01	0.002					
BNP	−0.399	0.144	0.007					

Adjusted $R^2 = 0.436$. β, standardized beta; part. cor., partial correlation coefficient.

Table 3 Univariate and multivariable predictors of serum EPO levels

EPO							
Variable	Univariate			Multivariable			
	B	SE	*P*-value	B	SE	β	*P*-value
Sex	0.03	0.233	0.6				
Age	0.027	0.6	0.233				
Hb	−0.052	0.019	0.007	−0.044	0.019	−0.233	0.025
NYHA	−0.06	0.031	0.061				
CRP	0.345	0.126	0.006	0.125	0.054	0.234	0.024

Adjusted $R^2 = 0.111$.

Discussion

The present study demonstrated that anaemia in CHF patients was not only independently related to impaired renal perfusion and blunted EPO production, but to an increased ECV as well. However, in contrast to our expectations, serum EPO levels were not directly related to renal perfusion.

The association between anaemia in CHF and impaired EPO production has been suggested previously.[3,7] Nevertheless, the presence of defective endogenous EPO production was not formally evaluated until recently. In a comprehensive retrospective analysis on the cause of anaemia in CHF patients, Opasich *et al.*[15] found that 50% of anaemic CHF patients showed evidence of impaired EPO production. Our data further substantiate these findings.

The relation between renal perfusion and EPO levels has been evaluated previously in two populations comprising 13 and 14 CHF patients.[16,17] In these studies, EPO production inversely correlated with RBF, ERPF, and renal oxygen delivery, suggesting that impaired renal oxygenation caused the elevated EPO levels. However, in our far larger cohort, these findings could not be reproduced. Although there was no relation between ERPF and EPO production, a univariate mild correlation between EPO production and GFR was observed, which might implicate that blunted EPO production results from impaired renal function and structural renal damage. Furthermore, circulating inflammatory cytokines and ACE-inhibitors can directly inhibit EPO production in the kidney and might contribute to the blunted EPO production.[18,19] Additionally, impaired GFR could attenuate the excretion of circulating erythropoiesis-inhibiting factors (e.g. AcSDKP), leading to enhanced plasma levels, as has been demonstrated in a haemodialysis population.[20]

The non-anaemic CHF patients displayed higher EPO levels and *O/P* ratios than reference subjects, as has been described previously. Elevated EPO levels were independently related to higher CRP levels, suggesting that elevated EPO production is directly related to an enhanced inflammatory state. Several pro-inflammatory cytokines have inhibitory effects on erythropoiesis and are established as the cause of anaemia associated with chronic inflammatory disease.[21] CHF is associated with enhanced expression of a variety of pro-inflammatory cytokines, possibly contributing to the development of anaemia.[6] In addition, we have recently demonstrated that anaemia in CHF could be partially explained by elevated levels of AcSDKP, a negative regulator of haematopoietic stem cells.[8] These circulating factors inhibit erythropoiesis and can eventually result in elevated EPO requirements. Indeed, although EPO production was blunted, the circulating EPO levels in anaemic CHF patients were not reduced but slightly elevated compared with non-anaemic patients. The slightly elevated EPO levels were however insufficient for the prevailing Hb, reflected by significantly impaired *O/P* ratio. Hence, anaemia in CHF does not result from the inability to produce EPO, but an inability to further increase baseline EPO production.

As expected, anaemic patients displayed elevated ECV, which was independently related to lower Hb levels. Impaired renal haemodynamics in CHF cause activation of RAS and vasopressin systems, resulting in salt and fluid retention and consequently increased ECV. Fluid retention in CHF can cause haemodilution, resulting in pseudo-anaemia, which carries even a worse prognosis

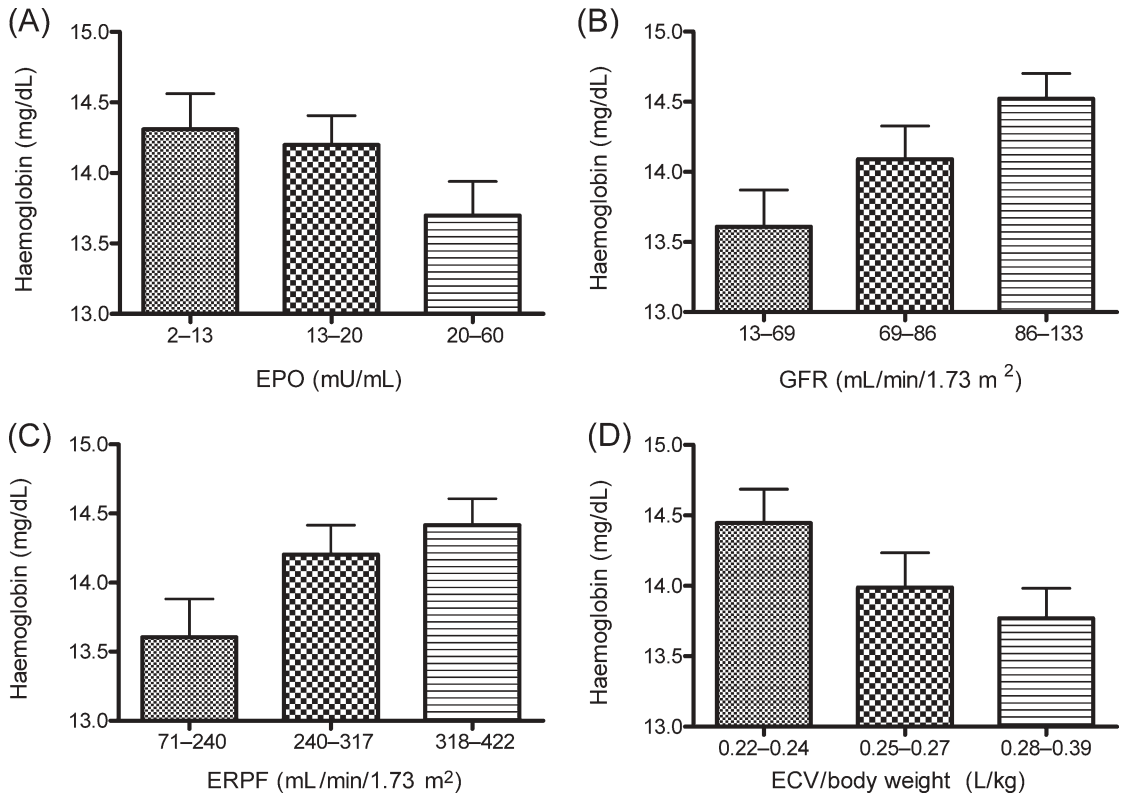

Figure 1 Relation between haemoglobin levels and EPO, GFR, ERPF, and ECV.

than true anaemia.[9] In the present study, anaemic subjects more frequently received diuretics but nonetheless displayed elevated ECV. Importantly, although fluid retention was related to anaemia, signs and symptoms of fluid retention were absent. Thus, haemodilution seems to precede the clinical presentation of fluid retention. Therefore, starting or increasing the dose of diuretics should be considered before starting with EPO treatment. Since the aetiology of anaemia in CHF seems multifactorial, the preponderant cause should be identified on an individual basis, for instance, by determining the *O/P* ratio or ECV in addition to regular diagnostic procedures.

Although the reduced renal perfusion, blunted EPO production, and elevated ECV could be the cause of anaemia in CHF, they could also be a consequence. Lower Hb levels can result in peripheral tissue hypoxia, causing vasodilatation and consequently reducing blood pressure.[22] This will result in activation of the RAS and further compromise of RBF by renal vasoconstriction and fluid retention. The compromised kidney seems unable to meet the increased demand, and anaemia ensues. The vicious cycle of CHF causing anaemia and anaemia causing further deterioration of CHF has been described as the cardiorenal anaemia syndrome.[23]

Thus, anaemia in CHF is directly related to an impaired haemodynamic state, compromising renal perfusion, attenuating EPO production, and increasing fluid retention. Therefore, improvement of cardiac function and cardiorenal haemodynamics would be the most rational approach for the treatment of anaemia in CHF. Additionally, administration of recombinant human EPO might break the vicious cycle by replenishing the insufficient EPO levels.[24] It is however uncertain whether supplementation of EPO in anaemic CHF patients will decrease morbidity and mortality, as anaemia might merely be a marker for impaired cardiac function. This will emerge from scheduled randomized clinical trails. The elevated ECV in our population suggests that concomitant meticulous correction of fluid overload might be feasible. Whether this will improve Hb and outcome in this population is however uncertain.

Limitations

Our study has limitations. Apart from the obvious cross-sectional design, the CHF population contained relatively few anaemic CHF patients and anaemic subjects had relatively mild anaemia. Therefore, our data might not be representative for more severe forms of anaemia and should be regarded as hypothesis generating.

Conclusion

Anaemia in CHF is not only independently associated with impaired renal perfusion and blunted EPO production, but to fluid retention as well.

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Conflict of interest: none declared.

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