Anemia, erythropoietin and iron in heart failure

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High serum erythropoietin levels are related to heart failure development in subjects from the general population with albuminuria: data from PREVEND.

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ABSTRACT

Aims

In patients with heart failure (HF), serum erythropoietin (EPO) levels are elevated and associated with disease severity and outcome. Whether endogenous EPO levels are prospectively associated with the development of HF or cardiovascular events in the general population is unknown.

Methods and Results

Serum EPO levels were measured at baseline in 6,686 subjects enrolled in the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study. Mean age (±SD) was 53±12 years, 49.8% were male, and median (IQR) EPO level was 7.7 (5.9–10.2) IU/L. During a median follow-up of 8.3 (7.7–8.8) years, 209 (3.1%) subjects were newly diagnosed with HF, 97 (1.5%) died of a cardiovascular cause and 386 (6.0%) subjects had a non-fatal cardiovascular event (277 cardiac events and 93 strokes). Each doubling of EPO level was multivariably associated with new onset HF (HR: 1.32 95% CI: 1.03–1.69, P=0.031). EPO levels showed interaction with urinary albumin excretion (P=0.006) and were only associated with HF in subjects with albuminuria (HR: 1.51, 95% CI: 1.13–2.03, P=0.005). There was an independent association of EPO levels with stroke in women (HR: 1.82, 95% CI: 1.24–2.65, P=0.002), but not in men. No association was observed for EPO levels with other cardiovascular events or cardiovascular mortality.

Conclusion

High serum EPO levels are independently associated with an increased risk of new onset HF in subjects with albuminuria. More research into the pathophysiological mechanisms linking EPO levels to HF is needed to understand this association.

INTRODUCTION

Erythropoietin (EPO) regulates the survival and proliferation of erythroid progenitor cells in the bone marrow.[1] In response to hypoxia, EPO production in the kidney is up-regulated to increase red blood cell production and restore oxygen delivery.[1] EPO production can be enhanced in response to both local hypoxia (e.g. impaired renal perfusion) and general hypoxia caused by anaemia, heart failure (HF), or pulmonary disease.[1]

In heart failure (HF) patients, EPO levels are elevated and correlate with a higher risk of hospitalization and mortality.[2,3] This might be due to the role of EPO as marker of hypoxia and inflammation[4], and thereby disease severity. EPO might, however, also play a causal role because high levels of the hormone have prothrombotic platelet-activating effects and are associated with hypertension.[5] Through these mechanisms, endogenous EPO is thought to be associated with adverse outcomes.[6] Two large trials investigating the correction of anaemia with exogenous EPO administration in subjects with chronic kidney disease and HF indeed found a higher incidence of stroke and venous thrombo-embolic events in the treated groups.[7,8]

Studies on the association of EPO levels with long-term outcome are currently restricted to patients with established HF and CKD,[2-4,9] and the very old (age >85yrs).[10] Data in the general population are lacking. Therefore, we aimed to assess the association of EPO levels with new onset HF and cardiovascular events in the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study, a large prospective, well characterized, observational cohort study.

METHODS

Study population

PREVEND, initiated in 1997 in Groningen, was a prospective, population based cohort study which enrolled 8,592 subjects. In short, all inhabitants of the city of Groningen aged 28-75 were sent a questionnaire and a vial to collect a first-morning-void urine sample. In total, 40,856 subjects (47.8%) returned a vial. Subjects with type 1 diabetes (defined as the use of insulin) and pregnant women (self reported) were excluded. The final cohort (n = 8,592) consisted of 6,000 subjects with an UAE ≥10mg/L and a randomly selected control group of 2,592 subjects with an UAE < 10 mg/L. We included 6,894 patients who completed the second survey because serum samples from baseline were absent. Patients with missing EPO measurements (n=117) or follow-up (n=31) and

patients with HF at baseline (n=60) were excluded, resulting in 6,686 subjects eligible for the analyses of new onset HF and cardiovascular mortality. Additionally, for the analyses of cardiovascular and cardiac events, we excluded subjects with a history of a myocardial infarction (n=195) or stroke (n=56), resulting in 6,435 subjects eligible for these analyses. Data of these subjects have been collected between 2001 and 2003. Data of death certificates of Statistics Netherlands are linked yearly to the PREVEND database. Clinical events (not leading to death) are registered using the Dutch PRISMANT database of hospital discharge diagnoses. In addition, subjects are seen every 3–4 year at the out-patient PREVEND facility. Details of the protocol are described elsewhere.[11] The PREVEND study has been approved by the medical ethics committee of the University Medical Center Groningen and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Erythropoietin and urinary albumin measurements

Fasting blood samples were obtained in the morning from all participants from 2001–2003. Serum was stored at -80 ºC and never thawed before assaying. Assaying was performed in 2013, after a storage time of ~10 years. Proteins are considered stable at -80 ºC when not exposed to freeze-thaw cycles but we cannot report on EPO levels immediately measured after sample obtainment. Serum EPO levels were measured using an immunoassay based on chemiluminescence (IMMULITE EPO assay ,DPC, Los Angeles, California).[12] The assay showed an intra-assay variability of 2.3–5.0%, an inter-assay variability of 4.1–9.5% and a lower detection limit of 0.16 IU/L. Urinary albumin concentration was measured by nephelometry, with a intra- and interassay variability of 2.2 and 2.6%, respectively, and a threshold of 2.3 mg/L (BNII, Dade Behring Diagnostics, Marburg, Germany).

Other clinical parameters

Anaemia was defined according to the World Health Organization criteria as a haemoglobin level <13.0 g/dL in men and <12.0 g/dL in women.[13] Diabetes mellitus was considered present when a subject was taking antidiabetic medication, had a nonfasting blood glucose >11.1 mmol/L or a fasting blood glucose >7.0 mmol/L.[14] Urinary albumin concentrations of 30 – 300 mg/L are normally considered to indicate microalbuminuria and concentrations of 10 – 30 mg/L are considered high-normal. We defined concentrations of ≥10 mg/L as albuminuria, in correspondence with the original study design. All participants were asked about their current and former smoking habits. Current smoking or quitted smoking within the previous year was defined as smoking. A history of myocardial infarction and/or cerebrovascular disease was considered present if a subject reported hospital admission because of these conditions. Left ventricular hypertrophy (LVH) was assessed with electrocardiography using the Cornell criteria: RaVL +

SV_3 (with 6mm added in women) multiplied by the QRS duration. LVH was defined as a value of >2440 mm*ms.

Heart failure

The identification method of new onset HF was discussed previously.[15] In short, patient files were checked in both hospitals located in Groningen, the University Medical Centre Groningen and Martini Hospital, for presence of HF at baseline and for new onset HF, by recording signs, symptoms and objective evidence of HF. Cases suspected of HF were identified using criteria in accordance to the Heart Failure Guidelines of the European Society of Cardiology[16] and evaluated by an endpoint adjudication committee of seven independent experts in the field of HF. Subsequently, two different experts validated each case. In case of disagreement, the committee made a joint decision. The aetiology and date of onset of HF was derived from clinical charts. In addition, HF was classified as HF with a reduced ejection fraction (HFrEF) or HF with a preserved ejection fraction (HFpEF) based on the left ventricular ejection fraction (LVEF) at the time of diagnosis. In accordance with the most recent HF guidelines,[16] HFpEF was defined as an LVEF >50%.

Cardiac and cardiovascular events and mortality

Information on hospitalizations was received from PRISMANT, the Dutch national registry of hospital discharge diagnoses. All data were coded according to the International Classification of Diseases, Tenth Revision (ICD-10) and the classification of interventions. Acute MI (ICD-10 code 410), acute and subacute ischemic heart disease (411), coronary artery bypass grafting or percutaneous transluminal coronary angioplasty were considered cardiac events. Stroke was defined as: subarachnoid haemorrhage (430), intra-cerebral haemorrhage (431), other intra-cranial haemorrhage (432) or occlusion or stenosis of the precerebral (433) or cerebral arteries (434). Cardiovascular events were defined as all cardiac events with the addition of strokes and vascular interventions as percutaneous transluminal angioplasty or bypass grafting of aorta and peripheral vessels. Data on cardiovascular mortality were retrieved through the municipal registry. To obtain cause of death, the death certificate was linked to the primary cause of death as coded by Statistics Netherlands (CBS).

Follow-up

Time to events was defined as the period from the date of the subject's visit until the date of first new onset HF, cardiovascular events, death, or 31 December 2010. If a person had moved to an unknown destination, the censor date was defined as the date on which the person was removed from the municipal registry.

Statistics

Data are presented as mean ± standard deviation (SD) when normally distributed, as median and interquartile range (IQR) when non-normally distributed, and as frequencies and percentages for categorical variables. Subjects in the lowest and highest 10% of EPO levels were studied separately as this cohort is derived from the general population and we expected to see the differences in characteristics and events in these groups. Differences between baseline variables were evaluated by the one-way analysis of variance, Kruskal-Wallis or chi-square test, when appropriate. For further analyses, skewed variables were transformed to a 2-log scale to achieve a normal distribution. Hazard ratios for the transformed variables should be interpreted as a relative risk if values were doubled (e.g. a change from 5 to 10 IU/L). To assess the best-fitting functional form for EPO levels and its association with new-onset HF, we performed fractional polynomial regression analyses. Cumulative incidence curves were constructed to estimate incidence of new onset HF and the Wald test was used to compare the incidence curves. Competing-risk regression analysis was used to assess whether EPO levels were associated with the studied endpoints. Death was considered a competing risk in all analyses. For the analyses of subcategories of HF (i.e. HFrEF versus HFpEF ischemic versus non-ischemic aetiology), developing HF of the other category was treated as a competing risk. Additionally, for the analyses of stroke and cardiac events, other cardiovascular events were considered as competing risks. The non-zero slope test by Therneau and Grambsch was used to test the proportional hazard assumption in the univariable competing-risk regression analyses. Interaction with EPO levels on outcome between all included variables was tested multivariably. Additionally, to assess whether the association with EPO levels was independent, the competing-risk regression model was adjusted for an established prediction model for HF consisting of presence of LVH, heart rate, systolic blood pressure, smoking, and levels of creatinine and fasting glucose.[17] Follow-up was truncated when <5% of the subjects were at risk, which was at 9.4 years. All P-values are two-sided, a P-value <0.10 was considered statistically significant for interaction analysis, for all other analyses a P-value <0.05 was considered statistically significant. All models and analyses were performed using STATA version 12.0 (StataCorp LP, College Station, Texas, USA) and GraphPad Prism version 6.01 (GraphPad Software, Inc., La Jolla, California, USA).

RESULTS

Baseline characteristics

The baseline characteristics of the 6,686 patients, stratified by EPO levels, are presented in **Table 1** and **Supplemental Table 1**. Mean (±SD) age was 53.4 ± 12.0 and 49.8% of the

subjects were male. Median (IQR) EPO levels were 7.7 IU/L (5.9–10.2); 7.6 IU/L (5.8–9.9) in men and 7.9 IU/L (6.0–10.6) in women. The subjects in the upper tenth percentile of EPO levels were more often women, were older and had a higher systolic blood pressure. A history of myocardial infarction, stroke or diabetes mellitus was more common in the subjects with the highest EPO levels, although the proportion of smokers was lower in this group. Also, levels of glucose, hs-CRP and UAE were higher in these subjects. Subjects with the highest EPO levels had a lower eGFR and were more often anaemic; 34.4% of the subjects with EPO levels in the upper tenth percentile were anaemic.

Table 1 – Baseline characteristics

		Erythropoietin			
Characteristic	**Total**	**< 10%**	**10 – 90%**	**> 90%**	**P-value**
Erythropoietin, min – max	0.6 – 750.0	0.6 – 4.5	4.5 – 13.7	13.8 - 750	
n	6,686	662	5,368	656	
Erythropoietin (IU/L)	7.7 (5.9 – 10.2)	3.7 (3.2 – 4.1)	7.8 (6.3 – 9.6)	16.7 (14.9 – 20.6)	N/A
Demography					
Age (years)	53.4 ± 12.0	51.6 ± 11.5	53.5 ± 12.0	54.9 ± 12.0	<0.001
Males (%)	49.8	52.1	50.8	39.0	<0.001
Waist circumference (cm)	92.1 ± 12.7	90.4 ± 11.6	92.0 ± 12.6	94.8 ± 14.5	<0.001
Systolic blood pressure (mmHg)	126.4 ± 18.7	125.2 ± 18.3	126.3 ± 18.6	128.8 ± 19.4	0.001
Heart rate (bpm)	68.4 ± 10.0	69.1 ± 9.6	68.2 ± 10.0	69.4 ± 10.7	0.003
LVH according to Cornell (%)	2.0	1.7	2.1	1.8	0.715
Baseline medical history					
Smoking or quit <1 year (%)	30.4	37.3	30.4	23.9	<0.001
Myocardial infarction (%)	2.9	1.1	3.0	4.3	0.002
Stroke (%)	0.9	0.4	0.8	2.3	<0.001
Diabetes mellitus (%)	8.0	6.7	7.5	13.8	<0.001
Laboratory values					
Glucose (mmol/L)	5.1 ± 1.2	5.0 ± 1.0	5.0 ± 1.1	5.3 ± 1.6	<0.001
Cholesterol (mmol/L)	5.4 ± 1.0	5.6 ± 1.1	5.4 ± 1.0	5.2 ± 1.0	<0.001
eGFR (mL/min/1.73m^2)	92.1 ± 17.0	94.3 ± 16.1	92.1 ± 16.8	90.1 ± 19.4	<0.001
UAE (mg/24h)	8.7 (6.1 – 15.8)	8.6 (6.2 – 14.8)	8.6 (6.0 – 15.6)	9.7 (6.4 – 20.5)	<0.001
hs-C-reactive protein (mg/L)	1.3 (0.6 – 3.0)	1.2 (0.5 – 2.6)	1.3 (0.6 – 3.0)	1.6 (0.7 – 3.7)	<0.001
Haemoglobin (g/dL)	13.7 ± 1.2	14.1 ± 1.1	13.8 ± 1.1	12.8 ± 1.6	<0.001
Anaemia (%)	9.3	2.9	7.1	34.4	<0.001
Ferritin (ug/L)	96 (48 – 172)	113 (57 – 189)	99 (51 – 174)	58 (15 – 142)	<0.001
MCV (fl)	90.5 ± 4.7	90.1 ± 4.0	90.7 ± 4.2	89.1 ± 7.7	<0.001

Values are given as means ± SD, medians (Q25 – Q75) or proportions (%). LVH = left ventricular hypertrophy, eGFR = estimated glomerular filtration rate, UAE = Urinary Albumin Excretion, MCV = Mean Corpuscular Volume. Hs-CRP is available in 5,563 (83.2%) of cases.

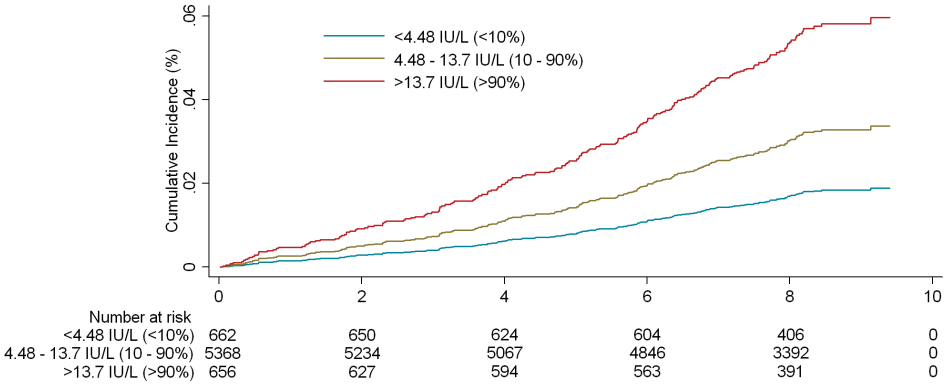

Figure 1 – Cumulative incidence of new onset HF. Groups are stratified by EPO levels in three groups: EPO levels <10%, between 10 and 90% or >90%. The difference between the cumulative incidence curves is assessed using the Wald test.

Table 2 – Competing-risk regression analyses for new onset HF.

	HR	95% CI	P-value
New Onset HF (n = 209)			
Doubling EPO unadjusted	1.34	1.16 – 1.55	<0.001
+ Age and sex	1.22	1.02 – 1.46	0.026
+ Haemoglobin, ferritin, TSAT, MCV and hs-CRP	1.34	1.05 – 1.72	0.021
+ HF risk model*	1.32	1.03 – 1.69	0.031

* HF risk model is adjusted for age, sex, haemoglobin, ferritin, TSAT, MCV, hs-CRP and additionally for waist circumference, eGFR, diabetes mellitus and other variables of the "Health ABC HF risk model" (presence of LVH, heart rate, systolic blood pressure, smoking, and levels of fasting glucose). Albumin levels were not available for adjustment.
Hs-CRP is available in 5,563 (83.2%) of cases.

Follow-up

New onset heart failure

During a median follow-up of 8.3 years (IQR 7.7–8.8), 209 (3.1%) subjects were newly diagnosed with HF. Subjects in the highest 10% of EPO levels were at the highest risk of new onset HF (**Figure 1**, Wald test: P<0.001 and **Supplemental Figure 1** for tertiles of EPO levels, Wald test: P=0.018). Using fractional polynomial analysis, the best fitting functional form for the association between new onset HF and EPO levels was obtained when EPO was logarithmically (log) transformed (log EPO vs. linear model, P<0.001). A linear rendering of the univariable association of EPO with new onset HF is presented as **Supplemental Figure 2**. A doubling of EPO levels was significantly associated with new onset HF in the univariable competing-risk regression analysis (hazard ratio (HR): 1.34, 95% confidence interval (CI): 1.16–1.55, P<0.001), see **Table 2**. This association remained significant after correction for haematological and inflammation parameters

and an established risk model for HF. A clear interaction was seen for UAE (P=0.006), and separate analyses were performed in subjects with and without albuminuria (i.e. UAE ≥10 mg/L), see **Table 3** and **Figure 2**. EPO levels were higher in subjects with albuminuria (8.0 IU/L [6.0–10.5] vs. 7.6 IU/L [5.8–10.0], P<0.001) and a higher proportion of subjects in this group developed HF (142 (5.1%) vs. 67 (1.7%), P<0.001). New onset HF was independently associated with EPO levels in albuminuric subjects (HR 1.51, 95% CI: 1.13–2.03, P=0.005), while an association in subjects without albuminuria was absent. No interaction was found between EPO levels and sex (P=0.396), eGFR (P=0.119), creatinine (P=0.744), haemoglobin (P=0.326) or any of the other included variables. Association between EPO and new onset HF with an ischemic aetiology vs. non-ischemic aetiology, and HF with a reduced ejection fraction (HFrEF) vs. HF with a preserved ejection fraction (HFpEF) are assessed separately (**Supplemental Figure 3***)*.

Table 3 – The association of EPO levels with new onset HF for subjects with and without albuminuria.

	No albuminuria (n = 3,845)			Albuminuria (n = 2,808)			Interaction
New Onset HF	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**	**P-value**
Doubling EPO unadjusted	0.90	0.64 – 1.27	0.559	1.42	1.23 – 1.63	<0.001	0.017
+ Age and sex	0.78	0.57 – 1.06	0.117	1.43	1.20 – 1.70	<0.001	0.002
+ Haemoglobin, ferritin, TSAT, MCV and hs-CRP	0.83	0.55 – 1.26	0.389	1.59	1.21 – 2.09	0.001	0.007
+ HF risk model*	0.79	0.52 – 1.21	0.282	1.51	1.13 – 2.03	0.005	0.006

* HF risk model is adjusted for age, sex, haemoglobin, ferritin, TSAT, MCV, hs-CRP and additionally for waist circumference, eGFR, diabetes mellitus and other variables of the "Health ABC HF risk model" (presence of LVH, heart rate, systolic blood pressure, smoking, and levels of fasting glucose). Albumin levels were not available for adjustment. Hs-CRP is available in 5,563 (83.2%) of cases. HF – Heart Failure, HR – Hazard Ratio, CI – Confidence Interval, EPO – Erythropoietin, TSAT – Transferrin saturation, MCV – Mean Corpuscular Volume, hs-CRP, high sensitivity C-Reactive Protein.

Cardiovascular and cardiac events and mortality

During follow-up, 97 subjects (1.5%) died of a cardiovascular cause, 386 (6.0%) had a non-fatal cardiovascular event and 277 (4.3%) had a non-fatal cardiac event. The cardiovascular events consisted, among others, of strokes (n=93), which we studied separately, since clinical trials suggested an increased risk of stroke associated with the use of exogenous EPO. Results of the survival analyses are presented in **Table 4**. Neither univariable, nor after correction for age and sex an association between EPO levels and cardiovascular mortality, cardiovascular events or cardiac events was observed. However, when stroke was studied separately, a significant univariable association was found (HR: 1.38, 95% CI 1.10–1.74, P=0.006). Multivariably, there was a trend to a 32% increased risk of stroke with doubling EPO levels (HR: 1.32, 95% CI 0.98–1.77, P=0.066). A significant interaction of EPO levels and sex (P=0.009) was found for the association with stroke, in which EPO levels were associated with stroke in women (HR: 1.82, 95%

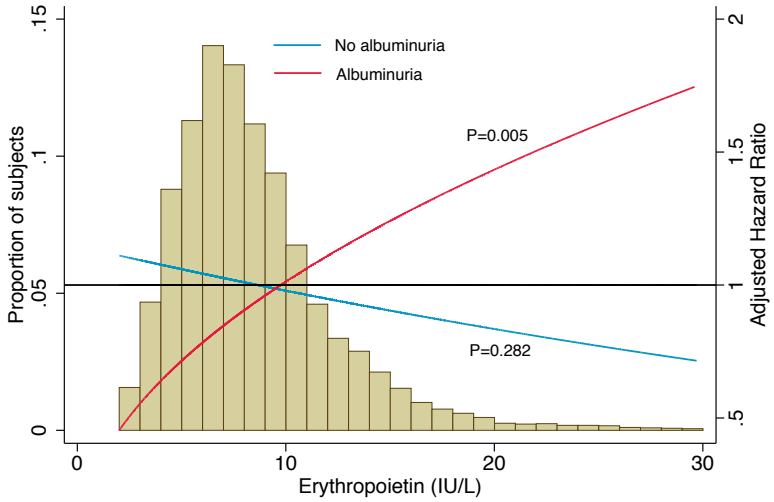

Figure 2 - Levels of erythropoietin and adjusted risk of new onset HF. Linear rendering of the adjusted hazard ratio of new onset HF, stratified for albuminuria. The proportion of subjects in the corresponding EPO range is depicted in the histogram on the background.

CI: 1.24–2.65, P=0.002), but not in men. No significant interactions were found of any of the other variables included in the analyses with EPO levels on either cardiovascular events, cardiac events or mortality.

Table 4 – Competing-risk regression analyses for cardiovascular events and mortality.

	HR	95% CI	P-value
Cardiovascular Events (n = 386)			
Doubling EPO unadjusted	1.06	0.92 – 1.22	0.428
+ Age and sex	0.99	0.85 – 1.15	0.888
Cardiac Events (n = 277)			
Doubling EPO unadjusted	0.93	0.79 – 1.10	0.386
+ Age and sex	0.88	0.74 – 1.04	0.131
Cardiovascular Mortality (n = 97)			
Doubling EPO unadjusted	1.24	0.97 – 1.59	0.083
+ Age and sex	1.06	0.80 – 1.41	0.678
Stroke (n = 93)			
Doubling EPO unadjusted	1.38	1.10 – 1.74	0.006
+ Age and sex	1.30	0.98 – 1.71	0.068
Model 1*	1.32	0.98 – 1.77	0.066

* Model 1 is adjusted for age, sex, smoking, heart rate, systolic blood pressure, haemoglobin and serum cholesterol and glucose. Subjects with a history of myocardial infarction or stroke were excluded from the analyses. HR – Hazard Ratio, CI – Confidence Interval.

DISCUSSION

In the current study, we show that EPO levels are independently associated with an increased risk of new onset HF in subjects with albuminuria, but not in subjects without. In addition, there is no association between EPO and cardiovascular or cardiac events, although a significant association between higher EPO levels and stroke was observed in women.

In patients with HF, EPO levels are generally high.[2,3] This could indicate bone marrow resistance to EPO, because of iron, vitamin B_{12} or folate deficiency,(18-20) or low grade inflammation, which is often present in these patients.[19] Impaired renal perfusion, fluid retention and general hypoxia are other conditions present in the majority of HF patients which could cause increased EPO levels.[2,21] These conditions, and associated high EPO levels, could also be present before subjects develop clinical signs and symptoms of disease. On the other hand, the prothrombotic platelet-activating effects of EPO and its association with hypertension could indicate a more causal relation of high EPO levels with disease progression.[5] In our cohort, subjects with the highest 10% of EPO levels had higher systolic blood pressures and higher levels of hs-CRP. Also, a higher prevalence of anaemia was seen in these subjects, which has been associated with an elevated cardiovascular risk as well.[22]

We found an association of EPO levels with new onset HF. This association was independent of other haematological parameters and cardiovascular risk factors. In particular, the association was independent of systolic blood pressure, the inflammation marker hs-CRP, haemoglobin and renal function. We recently showed a strong association between EPO levels and the components of the metabolic syndrome such as waist circumference, serum glucose and blood pressure, which are important cardiovascular risk factors.[23] However, EPO levels were associated with new onset HF independent of these variables. Interestingly, there was a clear interaction between UAE and EPO levels on outcome. EPO levels were related to new onset HF solely in subjects with albuminuria. High levels of albumin in the urine are traditionally considered to reflect renal disease. However, the association was independent of renal function, and we did not observe an interaction between renal function and EPO on new onset HF. Albuminuria is also strongly related to endothelial damage and high levels of hemostatic factors.[24] Subjects with albuminuria are therefore at high risk of developing cardiovascular disease.[25] High levels of EPO might be detrimental especially in these subjects, as EPO is thought to stimulate production and activity of platelets and increase levels of the hemostatic factors Von Willebrand factor and plasminogen activator inhibitor-1 (PAI-1)[5], high levels of which are also described in albuminuria. Of note, it is of debate if high EPO levels itself

or the associated low iron stores caused by increased erythropoiesis increase platelet levels.[26] We corrected our analyses for iron parameters (i.e. ferritin and MCV), and can therefore conclude that the association of high EPO levels with new onset HF we found is independent of the possible effects of iron on platelet levels.

Cardiovascular and cardiac events and mortality

If high EPO levels are related to increased thrombogenicity, one would expect to see an association between EPO levels and cardiovascular disease. We assessed cardiac and cardiovascular events and cardiovascular mortality separately and did not find an association of EPO levels with either of these endpoints.

The main regulator of EPO expression is hypoxia-inducible factor 1α (HIF-1α), an oxygen-sensitive transcription factor that enables the adaption to hypoxia by the transcription of, among others, EPO.[27] High levels of HIF-1α exert a cardio-protective effect closely linked to ischemic preconditioning.[28] In other words, short episodes of myocardial ischemia up-regulate HIF-1α, and thereby EPO, and may protect the heart against a subsequent acute myocardial infarction.[28,29] Thus, subjects with high EPO levels might be better protected for an acute ischemic event by ischemic preconditioning which might counterbalance the detrimental effects of EPO on thrombogenicity, resulting in the absence of an association between EPO levels and cardiovascular events. These effects are seen in experimental studies of acute ischemia.[28] On the long run, the detrimental effects of high EPO levels may prevail and long term upregulation of HIF-1α has been reported to induce HF and cardiomyopathy.[28] Although the underlying aetiology is still a subject of debate, several studies in mice showed chronic upregulation of HIF-1α to result in cardiomyopathy.[28,30-33] A first study by Minamishima et al. showed increased red blood cell production and venous congestion together with dilated cardiomyopathy after HIF-1α upregulation.[32] The cardiomyopathy in this case might be a consequences of the hyperviscosity and polycythemia, although the authors stated that direct effects of HIF-1α on cardiac function could not be excluded.[32] Direct cardiac effects were assessed in subsequent studies with cardiac specific HIF-1α upregulation, which showed development of cardiomyopathies as well.(30,31,33) Underlying mechanisms included reduced re-uptake of cytoplasmic calcium, intracellular glycogen and lipid accumulation and impaired mitochondrial functioning.(30,31,33) These direct effects of chronic HIF-1α upregulation on the heart might explain the differences in association between EPO and HF and more acute cardiovascular diseases.

When we assessed EPO levels in relation to strokes, we found a significant increase in risk with higher EPO levels in women. In clinical trials with erythropoiesis-stimulating agents (ESAs), higher rates of strokes compared to placebo were observed in both

sexes.[7,8] These trials, the TREAT and RED-HF, investigated the treatment of anaemia in patients with chronic kidney disease and HF, respectively.[7,8] It is thought that the central nervous system regulates its own response to hypoxia, as neurons and astrocytes are the main source of cerebral EPO and systemic EPO is supposed to be unable to cross the blood-brain barrier.[34] In this way, the brain might be less protected by ischemic preconditioning and the increased thrombogenicity results in a higher incidence of strokes. However, the number of subjects that encountered a stroke is limited (n=93) and therefore these results should be interpreted as hypothesis-generating.

Strengths and limitations

The large size of this prospective community-based cohort and detailed information on many covariates is a major strength of this study. Since none of the subjects used recombinant human EPO, this drug did not affect our results. Finally, because all blood samples were taken in the morning, the influence of circadian rhythm on EPO levels was minimized.

The lack of measurements of arterial oxygen, HIF-1α and circulating PAI-1 levels is a limitation, which prevented us from further exploring underlying physiologic mechanisms that could explain the relation between new onset HF, cardiovascular events and EPO levels. Second, as subjects from the PREVEND study are predominantly Caucasian, our results cannot be extrapolated directly to subjects from other ethnicities. EPO levels were measured in serum which was stored for approximately 10 years. Although proteins are considered to be stable at -80 ºC over long periods of time and samples were not previously freeze-thawed, we cannot exclude an effect of storage time on its levels. Endpoint adjudicated was performed for all HF cases but not for any of the other endpoints. Data regarding creatinin, eGFR, glucose and ferritin were missing in 2-5% of the subjects. However, results for EPO remained similar when these variables were excluded from the multivariable analyses. Finally, the PREVEND cohort is enriched for increased UAE. To take this into account, we assessed possible interactions between EPO levels and UAE on the endpoints discussed.

CONCLUSION

High serum EPO levels are independently associated with an increased risk of new onset HF in subjects with albuminuria. More research into the pathophysiological mechanisms linking EPO levels to HF is needed to understand this association.

CONFLICTS OF INTEREST

Drs. Klip received speaker fees from Vifor Pharma. Dr. Voors, Dr. van Veldhuisen and Dr. van der Meer received consultancy fees from Vifor Pharma. Dr. Voors and Dr. van der Meer received an unrestricted grant from Vifor Pharma. Dr. van Veldhuisen received board membership fees from Amgen Inc. and Vifor Pharma.

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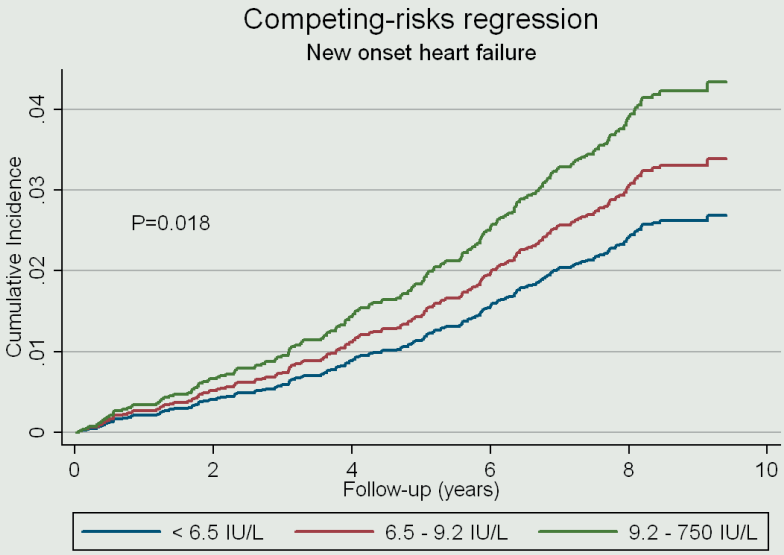
SUPPLEMENTARY MATERIAL

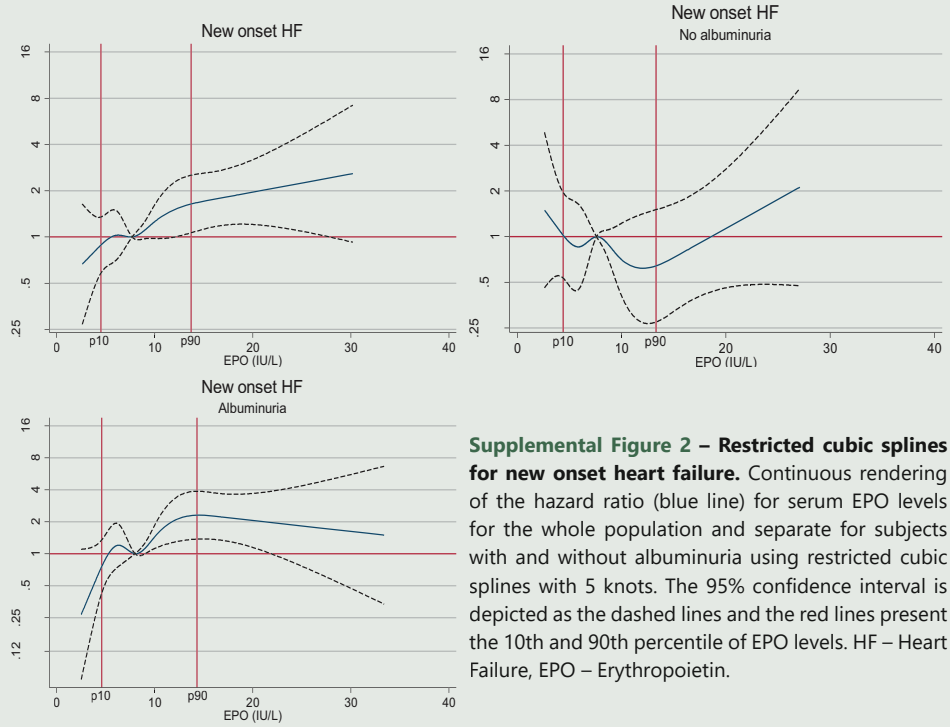
Supplemental Table 1 – Baseline characteristics.

		Erythropoietin			
Characteristic	**Total**	**<6.5**	**6.5 – 9.2**	**>9.2**	**P-value**
n	6,686	2231	2234	2221	
Erythropoietin (IU/L)	7.7 (5.9 – 10.2)	5.1 (4.3 – 5.9)	7.8 (7.1 – 8.4)	11.7 (10.3 – 14.4)	N/A
Demography					
Age (years)	53.4 ± 12.0	52.0 ± 11.6	53.1 ± 11.9	55.1 ± 12.4	<0.001
Males (%)	49.8	47.2	48.7	54.8	<0.001
Waist circumference (cm)	92.1 ± 12.7	90.9 ± 11.8	91.5 ± 12.6	94.0 ± 13.5	<0.001
Systolic blood pressure (mmHg)	126.4 ± 18.7	125.0 ± 17.8	125.7 ± 18.7	128.6 ± 19.4	<0.001
Heart rate (bpm)	68.4 ± 10.0	68.6 ± 9.9	68.2 ± 10.1	68.5 ± 10.0	0.330
LVH according to Cornell (%)	2.0	1.7	2.1	2.2	0.540
Baseline medical history					
Smoking or quit <1 year (%)	30.4	34.8	30.9	25.6	<0.001
Myocardial infarction (%)	2.9	2.1	3.0	3.6	0.007
Stroke (%)	0.9	0.9	0.7	1.2	0.260
Diabetes mellitus (%)	8.0	6.2	7.5	10.5	<0.001
Laboratory values					
Glucose (mmol/L)	5.1 ± 1.2	5.0 ± 1.0	5.0 ± 1.1	5.2 ± 1.4	<0.001
Cholesterol (mmol/L)	5.4 ± 1.0	5.5 ± 1.1	5.4 ± 1.0	5.3 ± 1.0	<0.001
eGFR (mL/min/1.73m^2)	92.1 ± 17.0	94.0 ± 15.8	92.5 ± 16.9	89.9 ± 18.1	<0.001
UAE (mg/24h)	8.7 (6.1 – 15.8)	8.5 (6.1 – 14.8)	8.6 (6.0 – 15.4)	9.2 (6.1 – 18.1)	<0.001
hs-C-reactive protein (mg/L)	1.3 (0.6 – 3.0)	1.2 (0.6 – 2.6)	1.3 (0.6 – 2.9)	1.5 (0.7 – 3.5)	<0.001
Haemoglobin (g/dL)	13.7 ± 1.2	14.0 ± 1.1	13.8 ± 1.1	13.4 ± 1.4	<0.001
Anaemia (%)	9.3	3.8	6.5	17.7	<0.001
Ferritin (ug/L)	96 (48 – 172)	110 (57 – 184)	99 (52 – 173)	82 (32 – 155)	<0.001
MCV (fl)	90.5 ± 4.7	90.3 ± 3.9	90.7 ± 4.1	90.5 ± 5.7	0.013

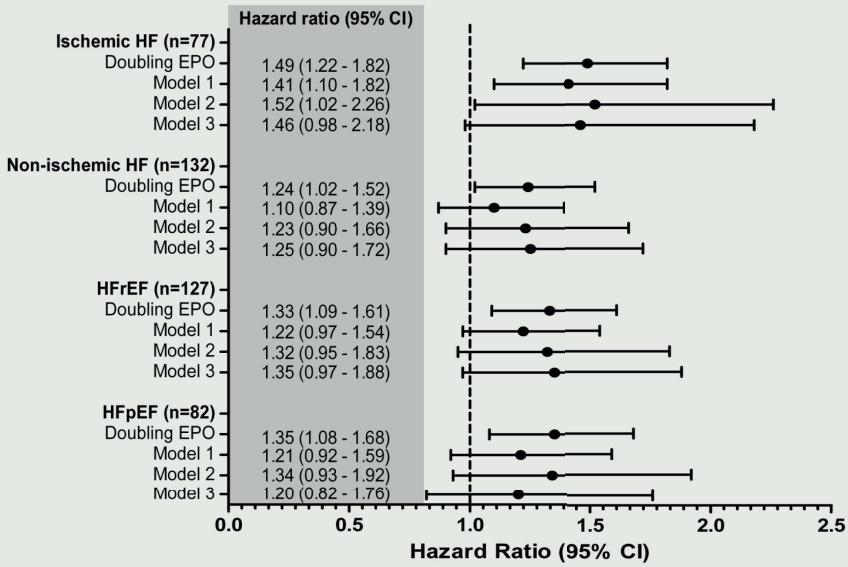
Values are given as means ± SD, medians (Q25 – Q75) or proportions (%). LVH = left ventricular hypertrophy, eGFR = estimated glomerular filtration rate, UAE = Urinary Albumin Excretion, MCV = Mean Corpuscular Volume. Hs-CRP is available in 5,563 (83.2%) of cases.



Supplemental Figure 1 – Cumulative incidence of new onset HF. Groups are stratified by tertiles of EPO levels. The difference between the cumulative incidence curves is assessed using the Wald test.



Supplemental Figure 2 – Restricted cubic splines for new onset heart failure. Continuous rendering of the hazard ratio (blue line) for serum EPO levels for the whole population and separate for subjects with and without albuminuria using restricted cubic splines with 5 knots. The 95% confidence interval is depicted as the dashed lines and the red lines present the 10th and 90th percentile of EPO levels. HF – Heart Failure, EPO – Erythropoietin.



Supplemental Figure 3 – Competing-risk regression analyses of new onset heart failure subgroups. Model 1: doubling EPO levels + age and sex. Model 2: model 1 + haemoglobin, ferritin, TSAT, MCV and hs-CRP. Model 3: model 2 + waist circumference, eGFR, diabetes mellitus and other variables of the "Health ABC HF risk model" (presence of LVH, heart rate, systolic blood pressure, smoking, and levels of fasting glucose). HF – Heart Failure, HFrEF – Heart Failure with reduced Ejection Fraction, HFpEF – Heart Failure with preserved Ejection Fraction, HR – Hazard Ratio, CI – Confidence Interval, EPO – Erythropoietin, TSAT – Transferrin saturation, MCV – Mean Corpuscular Volume, hs-CRP, high sensitivity C-Reactive Protein.