CHAPTER 4

HEMOGLOBIN LEVELS AND NEW ONSET HEART FAILURE IN THE COMMUNITY

IJsbrand T. Klip
Douwe Postmus
Adriaan A. Voors
Frank P.J. Brouwers
Ron T. Gansevoort
Stephan J.L. Bakker
Hans L. Hillege
Rudolf A. de Boer
Pim van der Harst
Wiek H. van Gilst
Dirk J. van Veldhuisen
Peter van der Meer

American Heart Journal (2015) 169, 94–101

ABSTRACT

Background

In established cardiovascular disease and heart failure (HF), low hemoglobin levels are associated with unfavorable outcome. Whether hemoglobin levels are associated with the development of new onset HF in the population is unclear. This study sought to investigate the relationship between hemoglobin levels and development of new onset HF in the community.

Methods

In 6744 patients from Prevention of REnal and Vascular ENd-stage Disease (PREVEND), a prospective, community-based, cohort study, we analyzed the relationship between hemoglobin levels and the risk of new onset HF.

Results

Mean age (±SD) was 53 ± 12 years, 49.8% was male and mean hemoglobin level was 13.7 ± 1.2 g/dL. During a median follow-up of 8.3 years [IQR 7.8–8.9], 217 subjects (3.2%) were newly diagnosed with HF. The association between hemoglobin levels and the risk for new onset HF was U-shaped ($P < 0.001$), remaining significant after full adjustment in a multivariable model with established cardiovascular risk factors ($P = 0.015$. Furthermore, an increased annual HF incidence was already observed in subjects with high-normal hemoglobin levels (men > 16 g/dL or women > 15 g/dL, $P = 0.041$), whereas on the other side of the distribution only severe anemia (men < 11 g/dL or women < 10 g/dL, $P = 0.018$) was associated with a higher annual incidence.

Conclusions

The impact of hemoglobin on the risk of new onset HF in the community is best described as U-shaped. Interestingly, higher hemoglobin levels, already within the high-normal range, are associated with an increased incidence. This was in contrast to anemia, where a higher annual HF incidence was only observed for severe anemia.

ABBREVIATIONS

BMI	=	Body mass index
CVD	=	Cardiovascular disease
HF	=	Heart failure
HFpEF	=	Heart failure with preserved ejection fraction
HFrEF	=	Heart failure with reduced ejection fraction
hs-CRP	=	high-sensitive C-reactive protein
LBBB	=	Left bundle branch block
LVH	=	Left ventricular hypertrophy
MI	=	Myocardial infarction
NO	=	Nitric oxide
UAE	=	Urinary albumin excretion
MCV	=	Mean corpuscular volume

INTRODUCTION

In patients with established cardiovascular disease (CVD) and heart failure (HF), the presence of anemia is associated with disease severity and affects outcome unfavorably.[1-3] In addition, in patients with end-stage renal disease, anemia is a risk factor for the development of left ventricular hypertrophy (LVH), and HF.[4,5] Despite its well-known role as a prognosticator in established CVD, studies addressing whether hemoglobin levels are associated with the development of new onset HF in the community are scarce. One study examined the role of hemoglobin as a predictor for new onset HF and found that anemia, based on ICD coding, was associated with the diagnosis and prognosis of new onset HF in the Medicare population.[6] However, this study only examined a selected cohort and had a short follow-up period, and therefore may not accurately represent the general population.

We therefore hypothesized that low hemoglobin levels would be associated with the development of HF, when adequately defined and modeled for potential confounding risk factors such as age, sex, blood pressure, albuminuria (as a marker for general endothelial dysfunction), smoking, history of myocardial infarction (MI), renal function and LVH. For these purposes, we used data obtained in the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study, a large prospective, well-characterized, observational cohort study with long-term follow-up.

METHODS

Study population

This study was performed using data of subjects from the PREVEND study. Details of the study protocol have been published elsewhere (www.prevend.org).[7] In brief, from 1997 to 1998, all inhabitants of the city of Groningen, The Netherlands, aged 28 – 75 years, were sent a questionnaire on demographics, disease history, smoking habits, use of medication, and a vial to collect an early morning urinary sample (n = 85,421). Of these subjects, 40,856 responded (47.8%). After the exclusion of subjects with type 1 diabetes mellitus (defined as the use of insulin), and pregnant women, subjects with an urinary albumin excretion (UAE) $\geq$ 10 mg/L (n = 6000) and a randomly selected control group with an UAE < 10 mg/L (n = 2592) completed the screening protocol and formed the baseline PREVEND cohort (n = 8592). For the current analyses we used data from the second survey, which took place between 2001 and 2003 (n = 6894), as hemoglobin measurements

were only available at this time period. Participants visited an outpatient clinic twice and were asked to perform two consecutive 24-h urine collections. We excluded 150 subjects because of missing hemoglobin values or other missing (baseline) values, leaving 6744 subjects for the current analysis. The PREVEND study was approved by the institutional medical ethics committee and conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent.

Definitions & calculations

Blood pressure was calculated as the mean of the last two out of ten measurements of the two study visits of the second survey, using an automatic Dinamap XL Model 9300 series. Hypertension was defined as systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or self-reported use of antihypertensive medication. The body mass index (BMI) was calculated as the ratio of weight and height squared (kg/m2), and obesity was defined as a BMI > 30 kg/m2. Hypercholesterolemia was defined as total serum cholesterol > 6.5 mmol/L (251 mg/dL) or cholesterol ≥ 5.0 mmol/L (193 mg/dL) if a history of myocardial infarction (MI) was present or when lipid-lowering medication was used. Type 2 diabetes mellitus was defined as a fasting glucose levels of ≥ 7.0 mmol/L (126 mg/dL), a non-fasting glucose level of ≥ 11.1 mmol/L (200 mg/dL), or the use of anti-diabetics. Smoking was defined as current smoking or smoking cessation within the previous year. Urinary albumin excretion was calculated as the average UAE in the two consecutive 24-h urine collections. Renal function was calculated as an estimate of the glomerular filtration rate by using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.8 A history of MI or stroke was reported as participant-reported hospitalization as a result of this condition. Anemia was defined according to the World Health Organization (WHO) criteria (hemoglobin < 13 g/dL for men and < 12 g/dL for women) and subdivided in moderate anemia (< 12 g/dL for men and < 11 g/dL for women) and severe anemia (< 11 g/dL for men and < 10 g/dL for women). High-normal hemoglobin levels were defined as hemoglobin > 16.0 g/dL for men and > 15.0 g/dL for women and elevated hemoglobin levels as hemoglobin > 17.0 g/dL for men and > 16.0 g/dL for women. Standard 12-lead electrocardiograms were recorded using the computer Modular ECG Analysis System. A left bundle branch block (LBBB) was defined as a QRS duration on ECG > 120 msec. Presence of LVH was defined using the Cornell criteria: RaVL + SV3 (with 6 mm added in women) multiplied by the QRS duration. A threshold of 2440 mm*ms was used to identify LVH.9

Analytical methods

Fasting blood samples were obtained in the morning from all participants from 2001–2003. Hematological measurements were performed in fresh venous blood. Aliquots of these samples were stored immediately at −80 °C until further analysis. Hemoglobin, hematocrit and mean corpuscular volume (MCV) were measured using a Coulter Counter STKS sum (Coulter Corporation, Miami, Florida, USA). Concentrations of total cholesterol, plasma glucose and serum creatinine were measured using standard methods. Urinary albumin concentration was determined by nephelometry, with a threshold of 2.3 mg/L and *intra*- and *interassay* coefficient of variation of 2.2 and 2.6%, respectively (BN II, Dade Behring Diagnostica, Marburg, Germany). High-sensitive C-reactive protein (hs-CRP) was also determined using nephelometry with a threshold of 0.175 mg/L and *intra*- and *interassay* coefficients of less than 4.4 and 5.7% respectively.

Heart failure and mortality

Follow-up for the present study was defined as the time between the first follow-up visit to the outpatient department and the date of new onset HF, death, or 1 January 2011. Subjects were censored on the date they moved to an unknown destination or at the last date of follow-up (1 January 2011), whichever date came first. Information on dates and causes of death for every participant was obtained from Statistics Netherlands10 and coded according to the 10th revision of the International Classification of Diseases. Participants with a new diagnosis of HF were identified using criteria described in the Heart Failure Guidelines of the European Society of Cardiology and an endpoint adjudication committee ascertained the diagnosis of HF, as described elsewhere.11 Additionally, HF was classified as either HF with a reduced (HFrEF) or preserved ejection fraction (HFpEF) based on the left ventricular ejection fraction at the time of diagnosis. To acknowledge the most recent trends in cut-off for HFrEF and HFpEF in accordance with the most recent HF guidelines,12 we set the cut-off for HFpEF at > 50%.

Statistical analyses

Baseline variables are expressed as means with standard deviation (SD) when normally distributed, as medians with interquartile range (IQR) when distribution is skewed, or as numbers and percentages when categorical. Inter-group differences were tested using the one-way analysis of variance (ANOVA) test, Kruskal-Wallis test or chi-square test, as appropriate. For further analyses, skewed variables were transformed to a 2-log scale to achieve a normal distribution. Risk estimates for the transformed variables should

be interpreted as the relative risk if values were doubled (e.g. a change from 10 to 20 mg/24h).

By design, the PREVEND study over-selected subjects with an elevated UAE (> 10 mg/L). To overcome this over-selection of subjects with UAE, a statistical weighted method was applied in all our regression analyses. This design-based approach allows our conclusions to be generalized to the general population.[13,14] Using this approach, the association of hemoglobin with traditional cardiovascular risk factors was examined by bootstrapping the linear regression model 1000 times. Bootstrapping was used to narrow the candidate set of univariable significant variables ($P < 0.10$) that were explanatory for hemoglobin. The bootstrap sample size was 6744 (size of the entire data set). Variables selected > 700 times were assumed to be accurate and included in the multivariable model.

Annual incidence rates for new onset HF, stratified by sex-adjusted hemoglobin groups, were assessed assuming a Poisson distribution. Subsequently, Cox regression analysis was conducted to assess the association between continuous hemoglobin levels and new onset HF. To preserve hemoglobin as a continuous variable in our analysis, multivariate fractional polynomials were employed to determine the best fitting functional form for hemoglobin and its relationship with new onset HF. The best fitting form was compared against the null model and a model that included hemoglobin as a linear term.[15] To account for death as a competing risk for new onset heart failure, a subjects' follow-up time was censored at the time of death whenever death occurred before the onset of HF. Cox regression analysis then yields valid results provided that the hazard ratios are interpreted as the covariate effects on the cause-specific hazard function of the transition from HF-free to new onset HF in a competing risks model with new onset HF and all-cause mortality as the two absorbing states.[16] To correct for possible confounding factors, analyses were consecutively adjusted for age and sex, 2 established HF risk prediction models,[17,18] and finally a multivariable model consisting of the following cardiovascular risk factors: age, sex, BMI, heart rate, smoking, the presence of diabetes, hypertension or hypercholesterolemia, a history of MI and/or stroke, presence of LVH on electrocardiogram, renal function (estimated glomerular filtration rate), and levels of hs-CRP and UAE. Finally, the Harrell´s C coefficient for multiple models and the incremental value of hemoglobin on this coefficient was reported. All reported probability values are two-tailed and a value of $P < 0.05$ was used as the level of statistical significance. Models and analyses were performed using STATA software version 11.0 (StataCorp LP, College station, Texas, USA) and R version 2.14.2 (R Foundation, Vienna, Austria.

RESULTS

Baseline characteristics of the 6744 subjects, stratified per sex-adjusted hemoglobin quintiles, are displayed in *Table 1*. Mean age was 53.3 (±12.1) years and 49.8% of the cohort were male. Mean hemoglobin concentration was 13.7 (± 1.2) g/dL (14.5 [± 1.0] in men vs. 13.0 [± 1.0] in women; $P < 0.001$), 504 subjects (7.5%) were mildly anemic, 129 subjects (1.9%) had moderate or severe anemia and 205 subjects (3.0%) had a high-normal or an elevated hemoglobin concentration. Per increasing quintile, patients were generally older, had a higher BMI, blood pressure, heart rate, and were more likely to have cardiovascular risk factors (i.e. obesity, smoking, hypertension and hypercholesterolemia). Concentrations of cholesterol, glucose, hs-CRP at baseline were also higher per increasing hemoglobin quintile, however renal function was worst in both lowest and highest hemoglobin quintile. A similar pattern was seen with UAE and the presence of LBBB on electrocardiogram, which was highest in both the lowest and highest hemoglobin quintile. Interestingly, no significant trend in LVH or history of MI was observed between hemoglobin quintiles. When comparing sex-adjusted hemoglobin groups, a trend in prevalence of subjects with LVH per increasing group was observed, whereas presence of LBBB and other cardiovascular risk factors were higher in the lower and upper groups (*Supplementary Table 1)*.

Factors associated with hemoglobin levels

Univariable analyses of the baseline variables showed a strong negative correlation between hemoglobin and age, and a positive correlation with male sex and cardiovascular risk factors, such as BMI, blood pressure, heart rate, smoking, and levels of cholesterol (*Supplementary Table 2*). In a bootstrapped model for hemoglobin, these variables remained highly selected (*Supplementary Table 3*). The multivariable adjusted association of these variables is presented in *Table 2*. There was no significant interaction between components in the multivariate model and hemoglobin concentrations.

Hemoglobin levels and risk of new onset heart failure

During a median follow-up of 8.3 years (IQR 7.8 – 8.9) 217 individuals (3.2%) were newly diagnosed with HF. The annual HF incidence was higher in subjects with high-normal and elevated sex-adjusted hemoglobin levels compared to subjects with normal hemoglobin levels (P = 0.041 and $P = 0.015$, respectively). No difference in new onset HF incidence between mild or moderate anemic subjects and subjects with normal hemoglobin levels

Table 1. Baseline characteristics stratified by sex-adjusted quintiles of hemoglobin (total population, n = 6744).*

Variables	Total	Q1	Q2	Q3	Q4	Q5	P-value for trend
n	*6744*	*1459*	*1335*	*1375*	*1353*	*1222*	*NA*
Hemoglobin (g/dL)	*13.7* ± 1.2	*12.4* ± 1.0	*13.3* ± 0.7	*13.7* ± 0.7	*14.2* ± 0.7	*15.2* ± 0.8	*NA*
Male sex (%)	*49.8*	*49.4*	*50.6*	*50.2*	*44.9*	*54.2*	*NA*
Clinical signs							
Age (years)	53.3 ± 12.1	52.9 ± 12.6	52.4 ± 12.3	52.7 ± 11.8	53.5 ± 11.4	55.1 ± 12.0	< 0.001
BMI (kg/m2)	26.7 ± 4.4	25.8 ± 4.4	26.2 ± 4.1	26.7 ± 4.5	27.2 ± 4.3	27.7 ± 4.2	< 0.001
Obesity (%)	19.0	13.8	14.8	19.8	20.8	26.8	< 0.001
Systolic BP (mmHg)	126.6 ± 18.8	123.0 ± 18.2	123.7 ± 18.0	125.9 ± 17.8	127.5 ± 18.1	133.6 ± 20.2	< 0.001
Heart rate (b.p.m.)	68.4 ± 10.0	66.8 ± 10.0	67.2 ± 9.7	68.3 ± 9.5	69.6 ± 10.0	70.7 ± 10.7	< 0.001
LVH (%)	1.3	0.8	1.7	1.0	1.3	1.6	0.35
LBBB (%)	2.3	2.7	2.1	1.5	1.7	3.8	0.55
Medical history (%)							
Smoking or quit < 1 year	30.4	24.3	28.2	28.0	33.7	39.1	< 0.001
MI	1.2	0.9	0.9	1.0	0.8	2.5	0.14
Stroke	0.9	1.2	0.5	1.0	0.1	1.4	0.81
Diabetes	8.0	8.7	6.8	7.7	8.6	8.2	0.06
Hypercholesterolemia	24.9	21.0	20.7	23.7	27.5	32.7	< 0.001
Hypertension	31.8	26.1	26.0	30.0	33.9	44.6	< 0.001
Laboratory measurements							
Hematocrit (%)	41 ± 4	37 ± 3	40 ± 2	41 ± 2	42 ± 2	45 ± 3	< 0.001
MCV (fL)	90.5 ± 4.7	88.9 ± 6.2	90.4 ± 4.3	90.8 ± 4.0	91.0 ± 3.9	91.4 ± 4.1	< 0.001
hs-CRP (mg/L)	1.36 (0.63 – 3.08)	1.21 (0.55 – 3.18)	1.22 (0.57 – 2.87)	1.25 (0.56 – 2.85)	1.50 (0.72 – 3.18)	1.58 (0.82 – 3.13)	< 0.001
Cholesterol (mmol/L)	5.4 ± 1.1	5.2 ± 1.0	5.3 ± 1.0	5.4 ± 1.0	5.6 ± 1.0	5.7 ± 1.1	< 0.001
Glucose (mmol/L)	5.1 ± 1.2	5.0 ± 1.3	5.0 ± 1.0	5.0 ± 1.1	5.1 ± 1.2	5.2 ± 1.2	< 0.001
Creatinine (umol/L)	84.8 ± 20.9	86.4 ± 32.9	84.0 ± 14.4	83.6 ± 14.9	83.8 ± 14.6	86.3 ± 20.2	0.34
eGFR (ml/min/1.73m2)	89.6 ± 17.8	89.9 ± 20.0	90.8 ± 17.3	91.0 ± 17.1	89.5± 16.7	87.5 ± 17.0	< 0.001
< 60 ml/min/1.73m2 (%)	5.7	7.1	5.4	5.0	4.5	6.7	0.42
UAE (mg/24h)	8.7 (6.1 – 15.8)	8.4 (6.0 – 15.1)	8.3 (6.1 – 15.3)	8.1 (5.8 – 14.1)	8.7 (6.1 – 15.4)	10.2 (6.6 – 22.6)	< 0.001

*Continuous variables are presented as mean ± standard deviation or as median (inter-quartile range when non-normally distributed. Binary categorical variables are presented as percentages. Abbreviations: BMI = Body mass index; BP = Blood pressure; ECG = Electrocardiogram; eGFR = Estimated glomerular filtration rate; hs-CRP = high-sensitive C-reactive protein; LBBB = Left bundle branch block; LVH = Left ventricular hypertrophy; MCV = Mean corpuscular volume; MI = Myocardial infarction; UAE = Urinary albumin excretion.

Table 2. Multivariate regression coefficients between cardiovascular risk factors and hemoglobin (variables selected after bootstrapping).

Variables	Coefficient	95% CI	T	P-value	Standardized beta
Age (per 10 years)	−0.049	−0.077 to −0.022	−3.47	0.001	−0.049
Male sex	1.405	1.344 to 1.466	45.41	< 0.001	1.135
BMI (per kg/m2)	0.022	0.015 to 0.029	5.99	< 0.001	0.090
Systolic BP (per 5 mmHg)	0.039	0.028 to 0.049	7.32	< 0.001	0.132
Heart rate (per 5 bpm)	0.042	0.027 to 0.057	5.40	< 0.001	0.072
Smoking	0.149	0.082 to 0.216	4.35	< 0.001	0.128
MCV (per fL)	0.047	0.037 to 0.057	9.61	< 0.001	0.176
Cholesterol (per mmol/L)	0.123	0.094 to 0.153	8.14	< 0.001	0.105

Abbreviations: CI = Confidence interval, for other abbreviations, see *Table 1*

was observed (*Figure 1*). However, when anemia became severe, annual HF incidence increased significantly compared to normal hemoglobin concentrations (P = 0.018). To further explore the association between new onset HF and hemoglobin, we modeled hemoglobin on a continuous scale. When adjusted for age and sex, fractional polynomial analysis revealed total hemoglobin to be significantly associated with the risk of new HF with a cubic shape (U-shaped) as best fitting functional form (*Table 3*). Additionally, the model with a cubic term for hemoglobin performed significantly better than a Cox model with a linear term (*P* < 0.001), suggesting that the association between hemoglobin and the risk of new onset heart failure is indeed best described by means of a cubic function. This association remained statistically significant when consecutively adjusted for multiple models, and a final multivariable model consisting of conventional cardiovascular risk factors (P = 0.015; *Table 3*). No interaction was found between hemoglobin levels, sex and the risk of new onset HF (P = 0.14) or between hemoglobin and renal function (P = 0.38). The correlation between hemoglobin and the risk for new onset heart failure, as obtained from the fully adjusted model, is depicted in Figure 2, with the nadir of risk around 13.7 g/dL for hemoglobin. No relative incremental value was observed when hemoglobin was added to each multivariable model (*Table 3*). Finally, to account for current smoking as a potential confounder, a sensitivity analysis in non-smokers was performed. The relationship between hemoglobin levels and new onset HF in non-smokers still remained best predictive when U-shaped (P = 0.003) and performed significantly better compared to the linear model (P = 0.004).

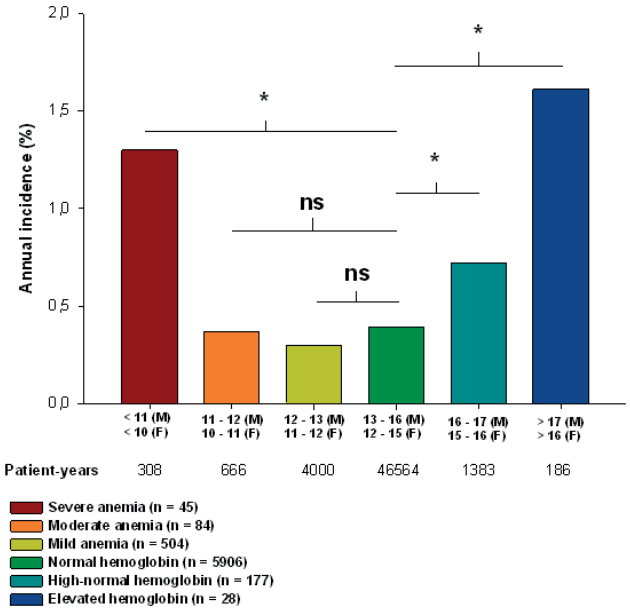

Figure 1. Annual incidence rates of new onset heart failure, stratified by sex-adjusted hemoglobin groups.
* *P* < .05

Heart failure with reduced or preserved ejection fraction

Of the 217 subjects who were newly diagnosed with HF, 132 were classified as HFrEF and 85 as HFpEF. Hemoglobin levels were significantly higher in subjects with HFrEF compared to HFpEF (14.2 g/dL vs. 13.8 g/dL, P = 0.031). Multivariable fractional polynomials, adjusted for the final model, revealed the relationship between total hemoglobin and development of HFrEF again to be best described as U-shaped (P = 0.005) and performed better than a linear model (P = 0.006). Total hemoglobin did not predict HFpEF (P = 0.85)

DISCUSSION

In this well-defined population-based cohort, we observed that the association between hemoglobin levels and the risk for new onset HF is best described as U-shaped and was independent of established conventional heart failure risk factors. Interestingly, higher hemoglobin levels, even within

Table 3. Cox regression of the association between hemoglobin levels the risk for new onset heart failure.

Hemoglobin*	Coefficient	SE	Z	P-value	P-value (vs. linear)	Harrell 's C (95%CI)	Harrell's C (95%CI) + HB	% change (P-value)
Age- and sex-adjusted				< 0.001	0.003	0.81 (0.78 – 0.83)	0.82 (0.79 – 0.84)	+ 0.78% (0.21)
HB3	−1.58	0.46	−3.47					
Log(HB)*HB3	2.60	0.58	4.46					
Model 1†				0.011	0.010	0.85 (0.83 – 0.88)	0.86 (0.84 – 0.88)	+ 0.51% (0.41)
HB3	−1.79	0.49	−3.65					
Log(HB)*HB3	2.79	0.66	4.23					
Model 2‡				0.003	0.001	0.84 (0.82 – 0.87)	0.85 (0.83 – 0.87)	+ 1.27% (0.13)
HB3	−2.41	0.50	−4.53					
Log(HB)*HB3	3.77	0.65	5.29					
Model 3§				0.015	0.005	0.87 (0.85 – 0.89)	0.88 (0.86 – 0.90)	+ 0.34% (0.54)
HB3	−1.87	0.50	−4.10					
Log(HB)*HB3	2.85	0.65	4.77					

Abbreviations: 95%CI = 95% confidence interval; HB = Hemoglobin; SE = Standard error, other abbreviations, see *Table 1*.

* In all equations; HB = HB/10.

† Model 1 is adjusted for age, sex and additionally for other variables of the *Health ABC HF risk model* (presence of LVH, heart rate, systolic blood pressure, history of MI, smoking and levels of creatinine and fasting glucose). Albumin levels were not available for adjustment.

‡ Model 2 is adjusted for age, sex and additionally for other variables of the *Framingham HF risk model* (presence of left ventricular hypertrophy, heart rate, systolic blood pressure, history of MI, diabetes and BMI). History of valve disease was not available for adjustment.

§ Model 3 is adjusted for age, sex and additionally for BMI, heart rate, smoking, the presence of diabetes, hypertension or hypercholesterolemia, a history of MI or stroke, LVH on electrocardiogram, renal function (eGFR), and levels of hs-CRP and UAE.

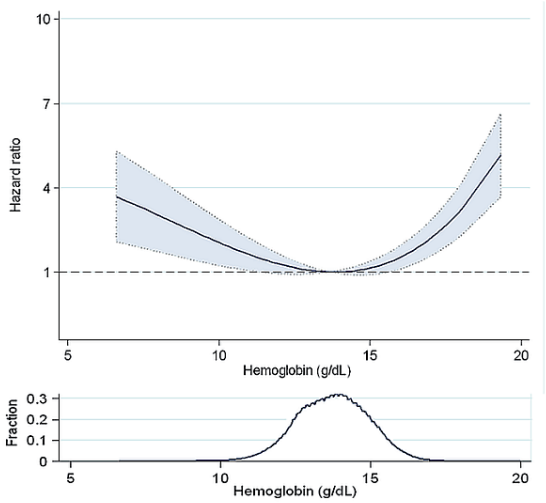

Figure 2. Hemoglobin levels and adjusted risk for new onset heart failure.

the high-normal range, were already associated with a clear increase in new onset HF incidence, whereas only severe anemia was associated with new onset HF compared to normal hemoglobin levels.

How do hemoglobin levels relate to the development of heart failure?

There are several mechanisms that could explain the associations of both low and high hemoglobin levels with the development of HF. First, as a presumed consequence of *lower* hemoglobin levels, lower blood viscosity, hypoxia and enhanced nitric oxide (NO) activity (hemoglobin is a powerful NO scavenger), vasodilatation and reduced vascular resistance may lead to an increased cardiac output. This might eventually result in progressive cardiac enlargement and LVH. It has been shown that cardiac output only increases when hemoglobin concentrations decline to 10 g/dL or less.19 When concentrations are below 10 g/dL, increased cardiac output and blood flow begin to compensate for tissue hypoxia.20 Indeed, we observed a higher heart rate and blood pressure in the sex-adjusted group with severe anemia compared to the moderate or mild anemia groups (*Supplementary Table 1)*. Furthermore, no differences in incidence of new onset HF between mild or moderate anemic subjects and subjects with normal hemoglobin concentrations were found. However, when hemoglobin concentrations decreased by 1 g/dL for both sexes, HF incidence increased compared to normal hemoglobin concentrations. Additionally, hazard estimates became

statistically significant around a hemoglobin level of 10 g/dL (*Figure 2*). Sandgren and colleagues found that the presence of anemia was associated with the diagnosis and prognosis of new onset HF in the Medicare population.6 However, this analysis only included patients 65 years or older and had a short-term follow-up, therefore may not accurately represent the general population. Second, the definition of anemia was based on ICD coding and not on measurements of hemoglobin, probably resulting in an underreporting of anemia.

Interestingly, on the other side of the distribution, *higher* hemoglobin levels were also associated with an increased risk for new onset HF. Several prior studies have linked elevated hematocrit (hemoglobin) values to hypertension, coronary heart disease and LVH.21-23 This may partially be explained by scavenging of NO by hemoglobin,24,25 but other factors causing erythrocytosis, including pulmonary disease and smoking, may also be involved. Higher hematocrit concentrations can lead to increased viscosity and reduced NO bioavailability, resulting in impaired flow-mediated dilatation and increased peripheral resistance. This is supported by a study by Madsen *et al,* showing that hemoglobin concentration was inversely correlated with radial artery luminal diameter.24 An increase in peripheral vascular resistance, the hallmark of established hypertension, alters wall stress in the left ventricle, which may consequently lead to LVH. Indeed, in the present study we observed a positive association between increasing hemoglobin concentrations and multiple accepted cardiovascular risk factors (e.g. blood pressure, smoking, total cholesterol). Although a positive association between hemoglobin levels and smoking was observed, sensitivity analysis revealed hemoglobin levels to remain associated with new onset HF in only non-smokers. Therefore, elevated hemoglobin concentrations, even within the high-normal range, might be associated with the development of heart failure through their role in the development of CVD and LVH compared to normal hemoglobin levels. Recent findings from the Framingham cohort also showed an increased risk between high-normal and elevated hematocrit and the development of HF.26 However, only a selected population consisting of non-anemic patients, aged 50 to 65 years old, was examined. Therefore, this study may not accurately reflect the general population. Furthermore, no significant association was found between lower hematocrit levels (25% to 42%) and incident HF in another substudy from the Framingham cohort.21 Our findings are in line with the latter observation, underscoring the notion that only severe anemia may be associated with new onset heart failure.

A novel finding of the current study was the U-shaped association between total hemoglobin levels and new onset HFrEF (and not HFpEF). This

may partially be explained by the fact that we observed more established cardiovascular risk factors, associated with HFrEF, in both the lowest and highest sex-adjusted hemoglobin groups (*Supplementary Table 1*). However, these results may be limited by the low number of cases (especially in the lowest and highest sex-adjusted hemoglobin groups) and should be interpreted with caution. Hence, the role of hemoglobin and the development of both HFrEF and HFpEF merits further investigation.

Clinical implications

Heart failure is a serious public health problem with an increasing prevalence that will continue to rise. Therefore, identifying patients at risk for HF is essential. The findings of the present study and recent literature suggest that hemoglobin (hematocrit) levels might aid in identifying patients at risk for CVD and new onset HF. Even in patients with high-normal hemoglobin concentrations, we observed higher sex-adjusted annual incidence rates compared to normal hemoglobin levels.

Biomarkers, like hemoglobin, can provide important information on disease etiology and clinical risk. In addition to prior observed associations with CVD risk and the presently described U-shaped relationship with new onset HF, hemoglobin is a widely available routine marker and a relatively inexpensive measurement, making it a potential marker for identifying patients at risk for new onset HF. Additionally, as suggested in a recent review by Holsworth and colleagues, regular blood donation may reduce an individuals' CVD risk through reduction in key hemorheological variables (e.g. blood viscosity).27 This potential benefit can also be seen in reduction of CVD risk associated with excessive iron, oxidative stress and inflammation.28 However, one might speculate if regular donation or more stringent cardiovascular risk management is needed in patients with high-normal or elevated hemoglobin levels. As with other studies that link lower or higher hemoglobin levels with outcome (in this case heart failure), the question remains whether hemoglobin levels itself or underlying diseases, causing these decreased or elevated levels, influence outcome. Therefore, more studies are warranted to confirm our observations.

Strengths and limitations

The large size of this prospective community-based cohort, long follow-up period, detailed information on many covariates and thorough validation of incident HF diagnosis, are strengths of this study. In addition, we used advanced statistical methods to compare shapes of the association of hemoglobin concentrations with new onset heart failure. Assuming the

linearity of the continuous predictor-outcome association without further investigation can lead to incorrect interpretation, particularly when the underlying relationship is not linear. The present study is limited by the fact that the subjects from the PREVEND study are predominantly Caucasian and our results can therefore not be extrapolated to subjects from other ethnicities. Second, the etiology of hemoglobin levels above or below the normal range is diverse and we do not have detailed information on specific etiologies (e.g. nutritional deficiencies deficiency, erythropoietin levels). Third, hemoglobin measurements were not available until the second survey. Additionally, additional markers relevant to risk and diagnosis (e.g. NT-proBNP and troponin levels) were only measured during the first survey and could therefore not be used for the present analyses. Finally, the PREVEND cohort is enriched for increased UAE. For this reason, we corrected for study design by conducting a design-based analysis. Furthermore, compared with the Framingham cohort, UAE was not higher in PREVEND and incidence of all-cause mortality and new onset HF is comparable to that of an unselected general population study.29

CONCLUSIONS

In conclusion, in this community-based cohort, hemoglobin levels showed a significant U-shaped relation with the risk of new onset HF. Even slightly elevated hemoglobin levels are already associated with a higher annual incidence of new onset HF. This in contrast to anemia, where a higher HF incidence was only observed for severe anemia.

REFERENCES

1. Lawler PR, Filion KB, Dourian T, Atallah R, Garfinkle M, Eisenberg MJ. Anemia and mortality in acute coronary syndromes: A systematic review and meta-analysis. *Am Heart J* 2013;165:143–153.e5.
2. Kengne AP, Czernichow S, Hamer M, Batty GD, Stamatakis E. Anaemia, haemoglobin level and cause-specific mortality in people with and without diabetes. *PLoS One* 2012;7:e41875.
3. Groenveld HF, Januzzi JL, Damman K, van Wijngaarden J, Hillege HL, van Veldhuisen DJ, van der Meer P. Anemia and mortality in heart failure patients a systematic review and meta-analysis. *J Am Coll Cardiol* 2008;52:818–827.
4. Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC, Barre PE. The impact of anemia on cardiomyopathy, morbidity, and and mortality in end-stage renal disease. *Am J Kidney Dis* 1996;28:53–61.
5. Sarnak MJ, Tighiouart H, Manjunath G, MacLeod B, Griffith J, Salem D, Levey AS. Anemia as a risk factor for cardiovascular disease in The Atherosclerosis Risk in Communities (ARIC) study. *J Am Coll Cardiol* 2002;40:27–33.
6. Sandgren PE, Murray AM, Herzog CA, Solid CA, Gilbertson DT, Collins AJ, Foley RN. Anemia and new-onset congestive heart failure in the general Medicare population. *J Card Fail* 2005;11:99–105.
7. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, Gans RO, Janssen WM, Grobbee DE, de Jong PE, Prevention of Renal and Vascular End Stage Disease (PREVEND) Study Group. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002;106:1777–1782.
8. Matsushita K, Mahmoodi BK, Woodward M, Emberson JR, Jafar TH, Jee SH, Polkinghorne KR, Shankar A, Smith DH, Tonelli M, Warnock DG, Wen CP, Coresh J, Gansevoort RT, Hemmelgarn BR, Levey AS, Chronic Kidney Disease Prognosis Consortium. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. *JAMA* 2012;307:1941–1951.
9. Smilde TD, Asselbergs FW, Hillege HL, Voors AA, Kors JA, Gansevoort RT, van Gilst WH, de Jong PE, Van Veldhuisen DJ. Mild renal dysfunction is associated with electrocardiographic left ventricular hypertrophy. *Am J Hypertens* 2005;18:342–347.
10. Statistics Netherlands. Available at http://www.cbs.nl/en-GB/menu/home/default.htm?Languageswitch=on [data specifically requested] (20 February 2013).
11. Brouwers FP, de Boer RA, van der Harst P, Voors AA, Gansevoort RT, Bakker SJ, Hillege HL, van Veldhuisen DJ, van Gilst WH. Incidence and epidemiology of new onset heart failure with preserved vs. reduced ejection fraction in a community-based cohort: 11-year follow-up of PREVEND. *Eur Heart J* 2013;
12. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Bohm M, Dickstein K, Falk V, Filippatos G, Fonseca C, Gomez-Sanchez MA, Jaarsma T, Kober L, Lip GY, Maggioni AP, Parkhomenko A, Pieske BM, Popescu BA, Ronnevik PK, Rutten FH, Schwitter J, Seferovic P, Stepinska J, Trindade PT, Voors AA, Zannad F, Zeiher A, ESC Committee for Practice Guidelines. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the

Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2012;33:1787–1847.

13. Linssen GC, Bakker SJ, Voors AA, Gansevoort RT, Hillege HL, de Jong PE, van Veldhuisen DJ, Gans RO, de Zeeuw D. N-terminal pro-B-type natriuretic peptide is an independent predictor of cardiovascular morbidity and mortality in the general population. *Eur Heart J* 2010;31:120–127.
14. Skinner CJ, Holt D, Smith TM. In: Editors: Analyses of Complex Surveys, 1989, Chichester, John Wiley and Sons, pp 195–198, ed.
15. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol* 1999;28:964–974.
16. Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. *Stat Med* 2007;26:2389–2430.
17. Butler J, Kalogeropoulos A, Georgiopoulou V, Belue R, Rodondi N, Garcia M, Bauer DC, Satterfield S, Smith AL, Vaccarino V, Newman AB, Harris TB, Wilson PW, Kritchevsky SB, Health ABC Study. Incident heart failure prediction in the elderly: the health ABC heart failure score. *Circ Heart Fail* 2008;1:125–133.
18. Kannel WB, D'Agostino RB, Silbershatz H, Belanger AJ, Wilson PW, Levy D. Profile for estimating risk of heart failure. *Arch Intern Med* 1999;159:1197–1204.
19. Varat MA, Adolph RJ, Fowler NO. Cardiovascular effects of anemia. *Am Heart J* 1972;83:415–426.
20. Metivier F, Marchais SJ, Guerin AP, Pannier B, London GM. Pathophysiology of anaemia: focus on the heart and blood vessels. *Nephrol Dial Transplant* 2000;15 Suppl 3:14–18.
21. Gagnon DR, Zhang TJ, Brand FN, Kannel WB. Hematocrit and the risk of cardiovascular disease—the Framingham study: a 34-year follow-up. *Am Heart J* 1994;127:674–682.
22. Kunnas T, Solakivi T, Huuskonen K, Kalela A, Renko J, Nikkari ST. Hematocrit and the risk of coronary heart disease mortality in the TAMRISK study, a 28-year follow-up. *Prev Med* 2009;49:45–47.
23. Sorlie PD, Garcia-Palmieri MR, Costas R,Jr, Havlik RJ. Hematocrit and risk of coronary heart disease: the Puerto Rico Health Program. *Am Heart J* 1981;101:456–461.
24. Madsen PL, Scheuermann Freestone M, Neubauer S, Channon K, Clarke K. Haemoglobin and flow-mediated vasodilation. *Clin Sci (Lond)* 2006;110:467–473.
25. Natali A, Toschi E, Baldeweg S, Casolaro A, Baldi S, Sironi AM, Yudkin JS, Ferrannini E. Haematocrit, type 2 diabetes, and endothelium-dependent vasodilatation of resistance vessels. *Eur Heart J* 2005;26:464–471.
26. Coglianese EE, Qureshi MM, Vasan RS, Wang TJ, Moore LL. Usefulness of the blood hematocrit level to predict development of heart failure in a community. *Am J Cardiol* 2012;109:241–245.
27. Holsworth R,Jr, Cho Y, Weidman JJ, Sloop G, St Cyr J. Cardiovascular benefits of phlebotomy: relationship to changes in hemorheological variables. *Perfusion* 2013;
28. Meyers DG, Jensen KC, Menitove JE. A historical cohort study of the effect of lowering body iron through blood donation on incident cardiac events. *Transfusion* 2002;42:1135–1139.
29. Velagaleti RS, Gona P, Larson MG, Wang TJ, Levy D, Benjamin EJ, Selhub J, Jacques PF, Meigs JB, Tofler GH, Vasan RS. Multimarker approach for the prediction of heart failure incidence in the community. *Circulation* 2010;122:1700–1706.

Table S1. Baseline characteristics according to sex-adjusted haemoglobin groups (total population, n = 6744).

Variables	Group 1 < 11 (M)/< 10 (F)	Group 2 < 12 (M)/< 11(F)	Group 3 < 13(M)/< 12(F)	Group 4 13–16(M)/12–15(F)	Group 5 16–17(M)/15–16(F)	Group 6 > 17(M)/> 16 (F)	P-value for trend
n	*45*	*84*	*504*	*5906*	*177*	*28*	*NA*
Hemoglobin (g/dL)	*9.2 ± 1.1*	*10.9 ± 0.5*	*11.9 ± 0.5*	*13.9 ± 0.9*	*16.1 ± 0.5*	*17.5 ± 1.3*	*NA*
Male sex (%)	*31.1*	*28.6*	*27.6*	*51.4*	*70.1*	*67.8*	*< 0.001*
Clinical signs							
Age (years)	53.9 ± 13.3	52.6 ± 12.9	51.4 ± 12.6	53.3 ± 12.0	57.4 ± 11.5	55.4 ± 11.2	< 0.001
BMI (kg/m2)	25.7 ± 4.1	26.6 ± 4.9	25.8 ± 4.8	26.7 ± 4.3	28.1 ± 3.8	28.2 ± 4.4	< 0.001
> 30 (%)	15.6	21.4	14.1	19.0	28.6	29.0	0.001
Systolic BP (mmHg)	121.9 ± 19.6	121.3 ± 18.3	120.5 ± 18.8	126.8 ± 18.6	137.9 ± 19.5	140.6 ± 17.5	< 0.001
Heart rate (b.p.m.)	68.9 ± 11.7	67.9 ± 9.9	67.4 ± 9.5	68.4 ± 10.0	72.2 ± 11.2	72.5 ± 9.6	< 0.001
LVH on ECG (%)	0	0	1.0	2.1	2.2	10.7	0.002
LBBB on ECG (%)	11.1	8.3	4.4	5.7	8.5	17.9	0.41
Medical history (%)							
Smoking or quit < 1 year	17.9	24.4	24.2	30.4	49.7	64.3	< 0.001
MI	6.7	7.1	2.4	2.8	6.2	3.6	0.66
Stroke	2.2	1.2	1.6	0.9	0.6	0	0.07
Diabetes	15.6	13.1	8.9	7.8	9.1	11.1	0.06
Hypercholesterolemia	13.3	20.2	17.1	25.3	35.8	40.7	< .001
Hypertension	33.3	31.0	21.7	32.0	51.4	59.3	< .001
Laboratory measurements							
Hematocrit (%)	29 ± 4	33 ± 2	36 ± 2	41 ± 3	47 ± 2	53 ± 9	< .001
MCV (fL)	75.6 ±10.4	84.7 ± 8.4	88.7 ± 5.9	90.1 ± 4.1	92.1 ± 4.7	92.8 ± 6.3	< .001
hs-CRP (mg/L)	1.52 (0.62 – 4.94)	1.02 (0.54 – 3.18)	1.24 (0.53 – 3.65)	1.35 (0.63 – 2.98)	1.90 (0.96 – 3.38)	2.27 (0.67 – 8.22)	0.020
Cholesterol (mmol/L)	5.7 ± 2.5	5.1 ± 1.1	5.1 ± 1.6	5.4 ± 1.0	5.6 ± 1.1	6.0 ± 1.2	< .001
Glucose (mmol/L)	5.0 ± 1.3	5.0 ± 1.0	5.0 ± 1.1	5.0 ± 1.1	5.3 ± 1.4	5.6 ± 1.3	< .001
Creatinine (umol/L)	108.3 ± 35.1	88.5 ± 24.4	83.4 ± 33.3	84.6 ± 16.2	88.6 ± 15.7	91.3 ± 21.9	< .001
eGFR (ml/min/1.73m2)	85.7 ± 30.3	87.8 ± 23.8	91.0 ± 20.6	89.7 ± 17.2	86. 0 ± 17.2	85.7 ± 20.7	< .001
< 60 (%)	15.6	9.5	7.7	5.4	6.8	10.7	0.005
UAE (mg/24h)	10.6 (6.0 – 42.7)	8.9 (6.4 – 14.4)	8.6 (5.8 – 15.2)	8.2 (6.1 – 15.5)	11.6 (7.8 – 43.9)	19.9 (9.8 – 50.5)	< .001

For abbreviations, see Table 1.

Table S2. Unadjusted regression coefficients between cardiovascular risk factors and haemoglobin concentrations.

Variables	Coefficient	95% CI	T	P-value
Age (per 10 years)	−0.063	−0.096 to −0.030	−3.72	0.001
Male sex	1.481	1.420 to 1.542	47.73	< 0.001
BMI (per kg/m2)	0.030	0.021 to 0.039	6.23	< 0.001
Systolic BP (per 5 mmHg)	0.093	0.082 to 0.104	16.26	< 0.001
Heart rate (per 5 bpm)	0.019	−0.001 to 0.038	1.94	0.05
LVH on ECG	0.250	−0.049 to 0.549	1.64	0.10
LBBB on ECG	0.530	0.356 to 0.704	5.97	< 0.001
Smoking	0.292	0.208 to 0.376	6.82	< 0.001
MI	0.455	0.230 to 0.682	3.96	< 0.001
Stroke	−0.001	−0.554 to 0.553	−0.01	0.98
MCV (per fL)	0.065	0.054 to 0.075	11.61	< 0.001
hs-CRP (per doubling)	−0.003	−0.029 to 0.023	−0.21	0.84
Cholesterol (per mmol/L)	0.160	0.123 to 0.197	8.49	< 0.001
Glucose (per mmol/L)	0.104	0.586 to 0.150	4.47	< 0.001
eGFR (per ml/min/1.73m2)	0.004	0.006 to 0.002	3.31	0.001
UAE (per doubling)	0.142	0.111 to 0.172	9.11	< 0.001

Abbreviations: CI = Confidence interval, for other abbreviations, see Table 1.

Table S3. Bootstrap analyses of univariable associated cardiovascular risk factors and hemoglobin concentrations (variable selected > 700 times were assumed to be accurate and included in the multivariate model).

Variables	Times selected
Age (per 10 years)	999
Male sex	1000
BMI (kg/m2)	1000
Systolic BP (per 5 mmHg)	1000
Heart rate (per 5 bpm)	1000
LBBB on ECG	250
Smoking	1000
MI	191
MCV (fL)	1000
Cholesterol (mmol/L)	1000
Glucose (mmol/L)	562
eGFR (ml/min/1.73m2)	145
UAE (per doubling)	376

For abbreviations, see Table 1.