Haemoglobin levels as a predictor for the occurrence of future cardiovascular events in adults–Sex-dependent results from the EPIC trial

European Journal of Internal Medicine xxx (xxxx) xxx

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Original article

Haemoglobin levels as a predictor for the occurrence of future cardiovascular events in adults–Sex-dependent results from the EPIC trial

Christian Jung [a,b], Ralf Erkens [a,*], Patricia Wischmann [a], Kerstin Piayda [c], Malte Kelm [a,b], Gunter Kuhnle [d]

[a] Division of Cardiology, Pulmonology and Vascular Medicine, Medical Faculty, University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Moorenstr. 5, Düsseldorf 40225, Germany
[b] Cardiovascular Research Institute Düsseldorf (CARID), Heinrich Heine University, Düsseldorf, Germany
[c] Department of Cardiology and Angiology, Universitätsklinikum Gießen und Marburg, Gießen 35391, Germany
[d] Department of Food and Nutritional Sciences, University of Reading, Reading, United Kingdom

ARTICLE INFO

Keywords:
Anemia
Healthy
Cardiovascular event
Myocardial infarction

ABSTRACT

Background: The impact of hemoglobin levels on the occurrence of future health events remains equivocal. Due to its integral role in human hemostasis, both, high and low hemoglobin levels may play a significant role in the development of future cardiovascular (CV) events in otherwise healthy adults.
Methods: Data from the European Prospective Investigation into Cancer (EPIC)-InterAct cohort was analyzed. In 13.648 individuals, physical activity, body mass index, family history of cardiovascular events, kidney function, smoking status, blood pressure and LDL levels were modelled to concomitant hemoglobin levels and correlated to the occurrence of clinically-overt cardiovascular events and death over a 21-year period. (Sex specific) cox regression analysis were used to develop hazard ratios (HRs) for CV events and all-cause mortality.
Results: Anemia (hemoglobin (HGB) levels < 13.0 g/dl in men and < 12.0 g/dl in non-pregnant women) were associated with an increased all-cause mortality in men but not in women (HR anemia in men 1.4 (1.2; 1.6)) p=<0.0001).This was particularly visible with increasing age. Various sex specific Cox regression models, accounting for several CV risk factors confirmed these results. The incidence of future CV events and myocardial infarction was significantly influenced by underlying HGB levels in men with increasing age but not in women.
Conclusion: The influence of HGB levels on future cardiovascular events is sex-dependent. In men, presenting with anemia at baseline, the overall survival probability was impaired with increasing age. After adjusting for several CV risk factors, abnormal hemoglobin levels could be identified as a risk factor for the development of clinically-apparent future CV events in men. None of these effects were observed in women.

1. Introduction

Anemia, defined by the World Health Organization (WHO) as hemoglobin (HGB) levels < 13.0 g/dl (9.0 mmol/L) in adult men and < 12.0 g/dl (7.5 mmol/L) in adult non-pregnant women [1], can be observed in nearly 25% of the global population [2]. Although it manifests across all age groups, the incidence tends to rise with increasing age [3]. Anemia is a frequent comorbidity in patients with acute and chronic cardiovascular (CV) and cerebrovascular (CBV) diseases, and a negative predictor for overall-survival in patients with chronic heart failure [4]. In the acute setting, anemia can be seen as a potential disease modifier: in patients presenting with acute coronary syndrome, anemia at baseline is an independent predictor of major bleeding events and excessive one-year mortality [5].

However, the correlation of HGB levels with long-term cardiovascular outcomes remains poorly understood, even though several population-based studies addressed this topic [6–9]. Considering the pathophysiological background, both decreased and increased HGB levels may be associated with altered clinical outcomes. HGB is the essential oxygen carrier in the blood, influences blood viscosity and can modify the innate immune response. On the one hand, chronic anemia results in cardiac dysfunction and ventricular remodeling with increased cardiac output and consecutive maladaptive left ventricular hypertrophy [10–12]. Low HGB levels lead to decreased blood viscosity, which is

* Corresponding author.
E-mail address: ralf.erkens@med.uni-duesseldorf.de (R. Erkens).

https://doi.org/10.1016/j.ejim.2023.08.004
Received 23 March 2023; Received in revised form 23 July 2023; Accepted 3 August 2023

an important stimulus for (neo)angiogenesis [13]. On the other hand, higher HGB levels, even within the normal range, are associated with thrombosis [14]. Furthermore, elevated hematocrit levels increase peripheral platelet activation and oxidative stress by releasing ADP in response to the accumulation of iron [15,16].

Therefore, a specific analysis of HGB levels in relation to long-term clinical prognosis is needed to understand a possible connection. In this population based study, we want to examine the influence of HGB levels, as well as the impact of hemoglobin kinetics, in healthy individuals on the occurrence of CV-, CBV- events and death, using data from a large community-based cohort study with a lengthy follow-up period. This investigation also focuses on sex-specific differences and includes sequentially adjusted Cox regression models for thorough investigation.

2. Methods

2.1. Data source

Data from the Norfolk cohort of the European Prospective Investigation into Cancer and Nutrition Norfolk (EPIC—Norfolk) prospective study was analyzed. The EPIC—Norfolk cohort recruited 30.447 individuals at baseline, aged between 40 and 75 years during the years 1993 and 1997. Willingness to participate and participation on general practice age sex registers in Norfolk were the only two inclusion criteria [17]. Of those, the majority attended a first health check, including the provision of non-fasting blood samples. Individuals provided self-reported information on their actual health status and behavioral habits (including diet and physical activity) on specifically designed questionnaires. Anthropometric data were collected by trained research nurses. Hemoglobin analysis was performed according to local laboratory standards.

During the follow-up period of 13-years, individuals were asked to attend several consecutive health examinations, including the provision of blood samples, and collection of behavioral and nutritional habits. Participating individuals provided written informed consent to linkage to medical record information and various follow-up studies with different disease endpoints. Cardiovascular diseases were defined as either self-reported angina, myocardial infarction, myocardial infarction, cerebrovascular disease, or peripheral vascular disease on a provided questionnaire at baseline and/or during follow-up or medical records containing the following International Classification of Disease (ICD)−10: I11-I79; ICD-9: 402–448 entities. Diabetes mellitus was defined as either the self-reported diagnosis of diabetes, provided on a questionnaires at baseline and/or during follow-up, glycated hemoglobin levels >6.5% (47.5 mmol/mol), or self-reported intake of antidiabetic medication.

2.2. Study endpoints: cardiovascular events and mortality

All participants were followed up for fatal and nonfatal CV events, and the present investigation includes events until 28 February 2018. Cause-specific hospital admission was collected via ENCORE (East Norfolk Commission Record, the hospital admissions database kept by the East Norfolk Health Commission) with the individuals' unique National Health Service (NHS) number as described and validated previously [18]. All subjects were flagged by the UK Office of National Statistics for death certification review, and trained nosologists coded death certificates according to the ICD. The primary endpoint of this study was the occurrence of the first CV event, defined as ICD-10: I10–I79; ICD-9: 410–448.

2.3. Ethical considerations

The research reported has adhered to the relevant ethical guidelines and was conducted in accordance with the Declaration of Helsinki (1975) and later amendments. Ethical approval was obtained from the Norwich Ethics Committee as described previously [19]. All participants provided written informed consent for data acquisition and linkage to medical records. The EPIC—Norfolk trial is registered at Clinicaltrials.gov (NCT03424668).

2.4. Statistical analysis

Data were analysed using R 3.6.2 (R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). A 5% threshold of statistical significance was used ($p < 0.05$). For the mortality analyses, participants were followed-up until either death or end of follow-up. Analysis regarding CV events were performed until either the first incidence of a cardiovascular event, death or end of follow-up. Hemoglobin or change in hemoglobin were used as continuous variable. Anemia, mild anemia (women: 12 mg/dl < HBG > 10 mg/dl; med: 13 mg/dl ⟨ HBG ⟩ 10 mg/dl) and anemia of inflammation, a subgroup of anemia with increased markers of inflammation (CRP levels > 5 mg/dl), were analyzed as binary variables. Sex-specific Cox regression models were computed for all outcomes of interest. Sequentially adjusted models were constructed: Model 1: Multivariable adjustment for BMI (continuous) and physical activity (categorical: inactive, moderately inactive, moderately active, active); Model 2: for parameters in Model 1 and health at baseline and family history of myocardial infarction or cerebrovascular disease; Model 3: parameters in Model 2 and estimated glomerulation filtration rate; Model 4: parameters of Model 2 and smoking status, blood pressure and LDL levels. Restricted cubic splines (3 knots, outer quantiles 0.1 and 0.9; using the rcs function) were used for all continuous variables.

3. Results

3.1. Baseline characteristics, and consecutive health checks

In 6033 men, and 7615 women, hemoglobin levels at the first and concomitant health checks were available. Mean age of the study population was 59.2 ± 9.2 years. Baseline plasma creatine levels were higher in men (93.7 ± 20.7 mmol/L) as compared to women (79.2 ± 19 mmol/L, $p < 0.001$). Positive family history for myocardial infarction (men: 37% vs. women: 39%) and CBV disease (men: 24% vs. women: 26%) were equally distributed between both sexes. Most of the study participants reported to be physical inactive (both sexes, each 32%). In 95% of cases, men did not present with anemia, whereas 10% of participating women met the WHO definition of anemia. Further information can be found in Table 1. The second and third health checks were performed 3.7 years and 13 years, respectively, after baseline data collection. Blood count and hemoglobin kinetics are tabulated in Table 2. All participants were followed up for fatal and non-fatal CV events, covering a median follow-up time of 21.2 (IQR 17.9; 22.7) years.

3.2. CV, CBV-events and mortality during follow-up

During a 21-year follow-up period, myocardial infarction was diagnosed in 942 (7%) participants. Men were significantly more often affected than women (men: 573 (10%) vs. women: 369 (5%), p=<0.0001). Stroke occurred in 1658 (12%) of participants, with no difference between sexes. All-cause mortality was higher in men as compared to women (men: 2423 (40%) vs. women 2208 (29%), $p = 0.0001$). In the overall population, 2% died due to myocardial infarction and 3% died from stroke. Further data can be found in Table 3.

3.3. Impact of anemia on all-cause mortality, the occurrence of myocardial infarction related death, and on the future incidence of myocardial infarction

Cox regression revealed that anemia had no impact on the survival

Table 1
Baseline characteristics of study population.

	All	Male	Female
n	13,648	6033	7615
Age [years]	59.2 (9.2)	59.6 (9.1)	58.9 (9.2)
BMI [kg/m^2]	26.2 (3.8)	26.5 (3.2)	26.1 (4.3)
Systolic Blood Pressure [mmHg]	135.2 (18.1)	137.2 (17.3)	133.6 (18.6)
No family history [n (%)]			
Myocardial infarction	8487 (62%)	3810 (63%)	4677 (61%)
Cerebrovascular disease	10,218 (75%)	4607 (76%)	5611 (74%)
Smoking status [n (%)]			
Current	1481 (10.9)	682 (11.3)	799 (10.5)
Ever	5313 (38.9)	2983 (49.4)	2330 (30.6)
Never	6071 (44.5)	1950 (32.3)	4121 (54.1)
No prevalence at baseline [n (%)]			
Myocardial infarction	13,197 (97%)	5679 (94%)	7518 (98%)
Cerebrovascular disease	13,443 (99%)	5912 (98%)	7531 (99%)
Diabetes mellitus	13,338 (98%)	5837 (97%)	7501 (99%)
Physical activity [n (%)]			
Inactive	4301 (32%)	1901 (32%)	2400 (32%)
Moderately inactive	3880 (28%)	1458 (24%)	2422 (32%)
Moderately active	3005 (22%)	1337 (22%)	1668 (22%)
active	2462 (18%)	1337 (22%)	1125 (15%)
Anemia (who classification) [n (%)]			
Non	12,578 (92%)	5728 (95%)	6850 (90%)
Mild	875 (6%)	284 (5%)	591 (8%)
Moderate	181 (1%)	16 (0.3%)	165 (2%)
Severe	14 (0.1%)	5 (0.1%)	9 (0.1%)
Red blood cell laboratory values			
Hemoglobin [g/dL]	13.8 (1.3)	14.6 (1.1)	13.2 (1.1)
Hematocrit [% blood volume]	40.5 (3.9)	42.7 (3.4)	38.7 (3.3)
Red blood cells [10^6/µL]	4.5 (0.4)	4.8 (0.4)	4.4 (0.4)
Mean corpuscular volume [fl]	89.1 (4.4)	89.6 (4.2)	88.8 (4.6)
Mean Corpuscular Haemoglobin Concentration [g/dL]	34.2 (1.2)	34.3 (1.1)	34.1 (1.1)
Red Blood Cell Distribution Width [µm]	13.3 (1.0)	13.3 (1.0)	13.3 (1.1)
Additional blood count results			
White blood cells [10^3/µL]	6.5 (1.8)	6.7 (1.9)	6.4 (1.7)
Lymphocytes [% total blood volume]	2.0 (0.8)	2.0 (0.8)	2.0 (0.7)
Granulocytes [% total blood volume]	4.0 (1.4)	4.1 (1.4)	3.9 (1.4)
Further laboratory analyses			
Plasma creatinine	85.7 (21.0)	93.7 (20.7)	79.2 (19.0)
HBA1C [%]	5.3 (0.8)	5.4 (0.9)	5.3 (0.8)
LDL [mmol/l]	3.94 (1.03)	3.89 (0.95)	3.97 (1.08)

probability of the overall cohort. The age- and sex-dependent survival chances for all patients are shown in Fig. 1. If the full study cohort was separated by sex, the same conclusion could be drawn for women but not for men (Fig. 1). In men, the overall survival probability of anemic study participants was impaired as compared to non-anemic men (HR 1.4 (95% CI 1.2; 1.6)).This effect was particularly visible with increasing age.

The incidence of myocardial infarction and related death rates were not influenced by HGB levels across all age groups and sexes. The impact of anemia on all-cause mortality, the occurrence of myocardial infarction related death, and on the future incidence of myocardial infarction is illustrated in Fig. 1.

Table 2
Population characteristics at second and third health check.

	All	Male	Female
n	13,648	6033	7615
Time to 2HC [years]	3.7 (0.7)	3.7 (0.7)	3.7 (0.7)
Time to 3HC [years]	13.0 (1.9)	13.0 (1.9)	13.0 (1.9)
Hemoglobin 2HC [g/dL]	14.0 (1.4)	14.8 (1.2)	13.5 (1.2)
Hemoglobin 3HC [g/dL]	13.7 (1.8)	14.3 (1.1)	13.2 (2.1)
Delta Hemoglobin 2 to 1HC [g/dL]	−0.3 (1.2)	−0.2 (1.2)	−0.3 (1.2)
Delta Hemoglobin 3 to 1HC [g/dL]	0.3 (2.0)	0.5 (1.1)	0.2 (2.4)
Blood count			
Red blood cells 2HC [10^6/µL]	4.5 (0.5)	4.7 (0.5)	4.4 (0.4)
Red blood cells 3HC [10^6/µL]	4.4 (0.4)	4.6 (0.4)	4.3 (0.3)
White blood cells 2HC [10^3/µL]	6.4 (1.9)	6.5 (2.0)	6.3 (1.8)
White blood cells 3HC [10^3/µL]	6.4 (2.7)	6.6 (3.4)	6.3 (2.0)
Lymphocytes 2HC [% total blood volume]	2.0 (1.0)	2.0 (1.1)	2.0 (0.9)
Lymphocytes 3HC [% total blood volume]	1.9 (2.2)	1.8 (3.0)	1.9 (1.3)
Granulocytes 2HC [% total blood volume]	3.8 (1.4)	3.9 (1.4)	3.7 (1.4)

HC: health check.

Table 3
Events.

	All	Male	Female
n	13,648	6033	7615
Disease incidence [n (%)]			
Myocardial infarction (MI)	942 (7%)	573 (10%)	369 (5%)
Stroke	1658 (12%)	775 (13%)	883 (12%)
Mortality [n (%)]			
All cause	4631 (34%)	2423 (40%)	2208 (29%)
Myocardial infarction* (MI)	259 (2%)	155 (3%)	104 (1%)
Stroke*	420 (3%)	173 (3%)	247 (3%)

3.4. Sex-specific sequential modeling: all-cause mortality, the incidence of MI/CV events and effect of hemoglobin kinetics over time

Sex-specific Cox regression models, adjusting for various CV risk factors, were computed. Low hemoglobin levels were associated with increased all-cause mortality in men. Low and especially high HGB level in men were associated with an increased risk for MI/CV rates in all three models. No significant correlation between HGB levels and all-cause mortality or the incidence of MI/CV events could be observed in woman (Fig. 2).

In the overall cohort, the incidence of MI was influenced by underlying HGB spectrum at the first health check up (Model 1: HR 1.13 (1.02; 1.25); Model 2: HR 1.14 (1.03; 1.26); Model 3: HR 1.14 (1.03; 1.26)). However, MI associated mortality was not affected. A change in HGB levels over time, i.e. from the first to the second health check up, was associated with excessive all-cause mortality in the overall study cohort (Model 1: HR 1.04 (1.01; 1.08); Model 2: HR 1.04 (1.00; 1.07); Model 3: 1.04 (1.00;1.08); Model 4: HR 1.04 (1.00:1.08)). For both, MI incidence and changing hemoglobin levels over time, no sex-specific correlation could be observed.

Specifically focusing on anemic study participants at baseline, sequential cox regression modeling revealed an increased all-cause mortality in the overall cohort (Model 1: HR 1.18 (1.06;1.31); Model 2: HR 1.17 (1.05; 1.31); Model 3: HR 1.16 (1.05; 1.30; Model 4: 1.21 (1.08;1.36)), with higher risk for men (Model 1: HR 1.44 (1.24; 1.67); Model 2: HR 1.42 (1.22; 1.65); Model 3: HR 1.40 (1.21; 1.63); Model 4: HR 1.39 (1.19;1.63)). Even mild anemic study participants show a significant increase mortality in the overall cohort (Model 1: HR 1.17 (1.04;1.32); Model 2: HR 1.16 (1.03;1.30); Model 3: HR 1.15 (1.02;1.29); Model 4: HR 1.20 (1.06;1.35)), with a higher risk for men (Model 1: HR 1.33 (1.14;1.56); Model 2: HR 1.31 (1.09;1.57); Model 3: 1.31 (1.11;1.53); Model 4: HR 1.32 (1.13;1.55)). Evaluating anemia combined with systemic inflammation, it revealed a higher risk of mortality in the overall cohort in each cox regression model (Model 1:

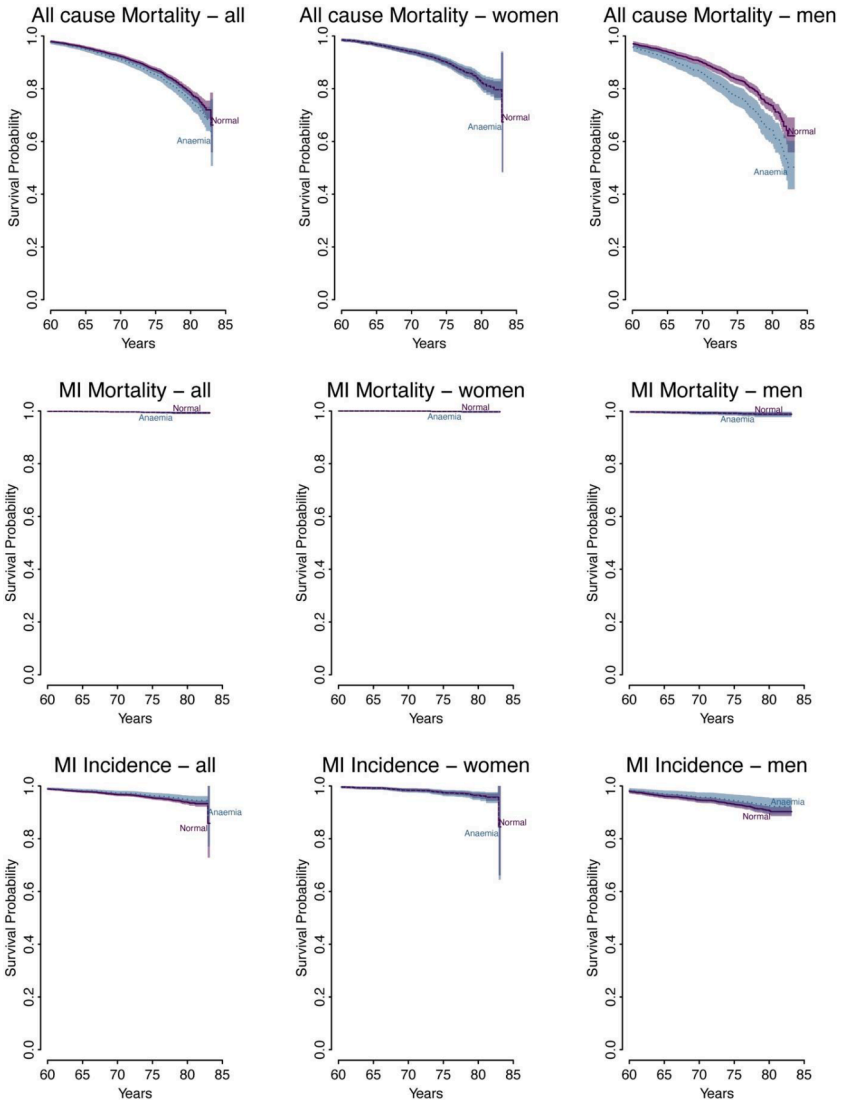

Fig. 1. Age-specific all cause mortality, myocardial infarction associated mortality and incidence for the study population: Anemia has no impact on all cause mortality in the overall-cohort and in women. In men presenting with anemia at the first health check up, the all-cause mortality is increased. Anemia did not have an impact on myocardial infarction related mortality nor on the incidence of myocardial infarction in both sexes.

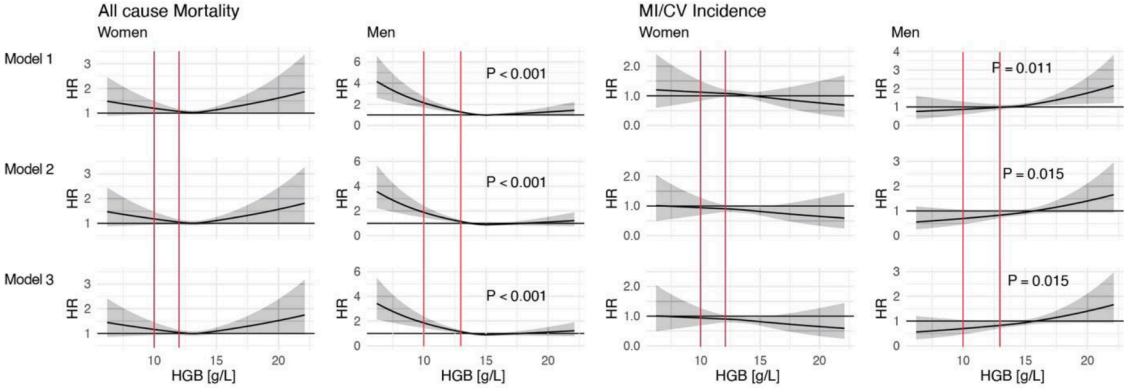

Fig. 2. After adjustment for different cardiovascular risk factors sequential cox regression models could show that, in men, all-cause mortality was significantly associated with the presence of lower HGB levels in all three models. Low and especially high hemoglobin levels were associated with an increased MI/CV incidence in men. None of these effects were observed in woman. Please note: plots are counterfactuals against an arbitrary baseline and should only be interpreted as an illustration of association. Red lines indicate the range of sex-specific normal HGB thresholds.

HR 1.19 (1.06;1.33); Model 2: HR 1.18 (1.05;1.32); Model 3: HR 1.17 (1.05;1.31); Model 4: HR 1.21 (1.09;1.36)), also with higher risk for men (Model 1: HR 1.4 (1.20;1.63); Model 2: HR 1.37 (1.18;1.61); Model 3: HR 1.36 (1.16;1.59); Model 4: HR 1.39 (1.19;1.63)). The incidence of MI/CV events and associated mortality did not show a correlation with anemia at baseline or changing hemoglobin levels over time. Further data is reported in Table 4.

Table 4

Association between baseline hemoglobin and change of hemoglobin between first and second health check. Adjusted Hazard ratios between bottom and top quartile and Wald's test for association (men and woman).

			All					Men					Women				
			1	1 (healthy)	2	3	4	1	1 (healthy)	2	3	4	1	1 (healthy)	2	3	4
HGB at baseline	Incidence	MI	**1.13 (1.02; 1.25)**	**1.17 (1.04; 1.31)**	**1.14 (1.03; 1.26)**	**1.14 (1.03; 1.26)**	1.04 (0.94; 1.23)	1.13 (0.93; 1.39)	1.19 (0.93; 1.52)	1.16 (0.95; 1.43)	1.16 (0.95; 1.43)	1.07 (0.87; 1.32)	1.11 (0.90; 1.37)	1.06 (0.84; 1.35)	1.10 (0.90; 1.36)	1.11 (0.90; 1.36)	1.04 (0.83; 1.31)
HGB at baseline	Mortality	MI	0.94 (0.79; 1.13)	1.05 (0.84; 1.31)	0.94 (0.79; 1.13)	0.96 (0.80; 1.16)	0.88 (0.72; 1.07)	0.90 (0.63; 1.27)	1.01 (0.64; 1.61)	0.94 (0.65; 1.35)	0.99 (0.68; 1.44)	0.86 (0.60; 1.24)	0.63 (0.35; 1.12)	0.57 (0.29; 1.11)	0.63 (0.35; 1.12)	0.62 (0.35; 1.11)	0.63 (0.35; 1.12)
HGB at baseline	Incidence	Stroke	1.03 (0.95; 1.10)	1.00 (0.93; 1.08)	1.03 (0.96; 1.11)	1.03 (0.96; 1.11)	0.99 (0.92; 1.07)	0.99 (0.85; 1.16)	0.97 (0.82; 1.14)	1.01 (0.86; 1.18)	1.00 (0.85; 1.17)	0.96 (0.82; 1.13)	0.91 (0.77; 1.07)	0.87 (0.73; 1.04)	0.91 (0.77; 1.07)	0.91 (0.77; 1.07)	0.87 (0.72; 1.03)
HGB at baseline	Mortality	Stroke	0.88 (0.76; 1.02)	**0.85 (0.72; 1.00)**	0.87 (0.75; 1.00)	0.87 (0.75; 1.00)	0.84 (0.72; 0.98)	0.81 (0.59; 1.11)	0.73 (0.52; 1.01)	0.80 (0.58; 1.10)	0.79 (0.58; 1.08)	0.81 (0.59; 1.13)	0.88 (0.64; 1.22)	0.87 (0.61; 1.24)	0.87 (0.63; 1.21)	0.87 (0.62; 1.20)	0.81 (0.57; 1.16)
HGB at baseline	Incidence	Combined	**1.06 (1.00; 1.13)**	1.05 (0.98; 1.12)	**1.07 (1.01; 1.14)**	**1.07 (1.01; 1.14)**	1.01 (0.95; 1.07)	1.08 (0.95; 1.23)	1.04 (0.90; 1.21)	1.11 (0.97; 1.27)	1.11 (0.97; 1.27)	1.05 (0.91; 1.20)	0.93 (0.81; 1.07)	0.91 (0.79; 1.06)	0.93 (0.81; 1.07)	0.93 (0.81; 1.07)	0.88 (0.76; 1.02)
HGB at baseline	Mortality	Combined	0.90 (0.81; 1.01)	0.91 (0.80; 1.04)	0.90 (0.80; 1.00)	0.90 (0.81; 1.01)	0.85 (0.76; 0.96)	0.85 (0.67; 1.07)	0.83 (0.63; 1.08)	0.86 (0.68; 1.09)	0.87 (0.68; 1.11)	0.83 (0.65; 1.06)	0.80 (0.60; 1.07)	0.79 (0.57; 1.07)	0.79 (0.60; 1.06)	0.79 (0.59; 1.05)	0.75 (0.55; 1.02)
HGB at baseline	Mortality	All cause	**0.94 (0.90; 0.98)**	**0.95 (0.90; 1.00)**	**0.94 (0.90; 0.98)**	**0.94 (0.90; 0.98)**	0.91 (0.87; 0.96)	**0.79 (0.72; 0.85)**	**0.78 (0.72; 0.85)**	**0.79 (0.73; 0.86)**	**0.80 (0.73; 0.87)**	0.80 (0.73; 0.87)	1.08 (0.99; 1.19)	1.09 (0.99; 1.20)	1.08 (0.99; 1.18)	1.08 (0.98; 1.18)	1.04 (0.94; 1.14)
HGB change (first to second check up)	Incidence	MI	1.04 (0.95; 1.13)	1.00 (0.91; 1.10)	1.03 (0.95; 1.12)	1.03 (0.95; 1.12)	0.99 (0.90; 1.08)	1.00 (0.90; 1.12)	0.97 (0.85; 1.10)	1.00 (0.89; 1.11)	1.00 (0.89; 1.11)	0.97 (0.87; 1.09)	1.09 (0.96; 1.24)	1.04 (0.90; 1.20)	1.07 (0.94; 1.22)	1.07 (0.94; 1.22)	1.00 (0.87; 1.16)
HGB change	Mortality	MI	1.02 (0.86; 1.21)	1.03 (0.87; 1.22)	1.01 (0.85; 1.20)	1.01 (0.86; 1.20)	0.93 (0.76; 1.13)	0.96 (0.79; 1.17)	0.97 (0.78; 1.20)	0.95 (0.78; 1.16)	0.95 (0.78; 1.16)	0.83 (0.64; 1.07)	1.18 (0.83; 1.66)	1.16 (0.83; 1.62)	1.15 (0.81; 1.62)	1.15 (0.81; 1.63)	1.15 (0.79; 1.67)
HGB change	Incidence	Stroke	1.06 (1.00; 1.13)	1.04 (0.97; 1.11)	1.06 (0.99; 1.13)	1.06 (0.99; 1.13)	1.05 (0.99; 1.12)	1.09 (0.99; 1.20)	1.06 (0.96; 1.17)	1.10 (1.00; 1.21)	1.09 (0.99; 1.20)	1.09 (0.99; 1.19)	1.01 (0.92; 1.11)	0.99 (0.89; 1.10)	1.00 (0.91; 1.10)	1.00 (0.91; 1.10)	1.01 (0.91; 1.11)
HGB change	Mortality	Stroke	1.07 (0.95; 1.20)	1.08 (0.95; 1.22)	1.06 (0.94; 1.20)	1.06 (0.94; 1.20)	1.07 (0.95; 1.21)	1.09 (0.89; 1.32)	1.08 (0.90; 1.29)	1.08 (0.88; 1.32)	1.07 (0.88; 1.30)	1.10 (0.91; 1.33)	1.04 (0.88; 1.23)	1.07 (0.89; 1.28)	1.04 (0.88; 1.23)	1.03 (0.87; 1.23)	1.06 (0.88; 1.27)
HGB change	Incidence	Combined	1.04 (0.99; 1.10)	1.02 (0.96; 1.08)	1.04 (0.98; 1.09)	1.04 (0.98; 1.09)	1.02 (0.96; 1.08)	1.05 (0.98; 1.13)	1.02 (0.94; 1.11)	1.05 (0.98; 1.13)	1.05 (0.97; 1.13)	1.03 (0.96; 1.11)	1.01 (0.93; 1.10)	0.99 (0.90; 1.08)	1.00 (0.92; 1.09)	1.00 (0.92; 1.09)	0.99 (0.90; 1.08)
HGB change	Mortality	Combined	1.05 (0.96; 1.16)	1.06 (0.96; 1.17)	1.05 (0.95; 1.15)	1.05 (0.95; 1.15)	1.03 (0.93; 1.14)	1.03 (0.90; 1.18)	1.03 (0.90; 1.18)	1.02 (0.89; 1.16)	1.01 (0.89; 1.16)	1.00 (0.87; 1.14)	1.07 (0.92; 1.25)	1.09 (0.93; 1.29)	1.06 (0.91; 1.24)	1.06 (0.91; 1.24)	1.08 (0.92; 1.27)
HGB change	Mortality	All cause	**1.04 (1.01; 1.08)**	**1.05 (1.01; 1.09)**	**1.04 (1.00; 1.07)**	**1.04 (1.00; 1.08)**	**1.04 (1.00; 1.08)**	1.04 (0.98; 1.09)	1.05 (0.99; 1.12)	1.03 (0.98; 1.08)	1.03 (0.98; 1.09)	1.04 (0.99; 1.09)	1.04 (0.99; 1.10)	1.04 (0.98; 1.10)	1.04 (0.99; 1.10)	1.04 (0.99; 1.10)	1.04 (0.98; 1.10)
Anaemic	Incidence	MI	0.86 (0.66; 1.12)	0.76 (0.56; 1.05)	0.82 (0.63; 1.06)	0.82 (0.63; 1.07)	0.95 (0.72; 1.24)	0.80 (0.55; 1.18)	0.69 (0.42; 1.12)	0.74 (0.50; 1.09)	0.75 (0.51; 1.10)	0.87 (0.59; 1.28)	1.05 (0.72; 1.52)	0.94 (0.62; 1.43)	1.03 (0.71; 1.50)	1.04 (0.72; 1.50)	1.15 (0.78; 1.68)
Anaemic	Mortality	MI	1.08 (0.67; 1.73)	0.70 (0.36; 1.37)	1.03 (0.64; 1.65)	1.01 (0.63; 1.63)	1.19 (0.73; 1.94)	1.11 (0.58; 2.12)	0.84 (0.34; 2.08)	1.01 (0.53; 1.93)	0.98 (0.51; 1.89)	1.15 (0.60; 2.22)	1.10 (0.56; 2.20)	0.57 (0.21; 1.57)	1.10 (0.55; 2.19)	1.09 (0.55; 2.17)	1.25 (0.60; 2.60)
Anaemic	Incidence	Stroke	1.01 (0.84; 1.22)	1.01 (0.83; 1.23)	1.00 (0.83; 1.20)	1.00 (0.83; 1.21)	1.06 (0.88; 1.29)	1.09 (0.82; 1.45)	1.01 (0.73; 1.41)	1.06 (0.80; 1.42)	1.08 (0.81; 1.44)	1.18 (0.88; 1.58)	1.02 (0.80; 1.30)	1.07 (0.83; 1.38)	1.01 (0.79; 1.29)	1.01 (0.79; 1.29)	1.02 (0.79; 1.31)
Anaemic	Mortality	Stroke	1.21 (0.86; 1.69)	1.17 (0.81; 1.71)	1.21 (0.86; 1.70)	1.21 (0.86; 1.70)	1.21 (0.84; 1.73)	1.51 (0.88; 2.58)	1.43 (0.74; 2.75)	1.51 (0.88; 2.58)	1.54 (0.90; 2.65)	1.46 (0.83; 2.57)	1.09 (0.70; 1.69)	1.12 (0.70; 1.77)	1.10 (0.71; 1.71)	1.11 (0.71; 1.72)	1.06 (0.66; 1.71)
Anaemic	Incidence	Combined	0.94 (0.80; 1.10)	0.93 (0.78; 1.11)	0.91 (0.78; 1.07)	0.92 (0.78; 1.07)	1.00 (0.85; 1.18)	0.92 (0.72; 1.17)	0.90 (0.68; 1.19)	0.87 (0.68; 1.11)	0.88 (0.69; 1.12)	0.99 (0.78; 1.27)	1.05 (0.85; 1.29)	1.05 (0.84; 1.31)	1.04 (0.84; 1.29)	1.04 (0.84; 1.28)	1.07 (0.86; 1.33)
Anaemic	Mortality	Combined	1.16 (0.88; 1.52)	1.01 (0.73; 1.41)	1.15 (0.87; 1.51)	1.14 (0.87; 1.51)	1.21 (0.90; 1.61)	1.32 (0.87; 1.99)	1.16 (0.68; 1.97)	1.28 (0.84; 1.94)	1.28 (0.85; 1.95)	1.34 (0.88; 2.05)	1.09 (0.75; 1.58)	0.96 (0.63; 1.46)	1.10 (0.76; 1.59)	1.09 (0.75; 1.58)	1.11 (0.75; 1.66)
Anaemic	Mortality	All cause	**1.18 (1.06; 1.31)**	**1.13 (1.00; 1.27)**	**1.17 (1.05; 1.31)**	**1.16 (1.05; 1.30)**	**1.21 (1.08; 1.36)**	**1.44 (1.24; 1.67)**	**1.41 (1.19; 1.67)**	**1.42 (1.22; 1.65)**	**1.40 (1.21; 1.63)**	**1.39 (1.19; 1.63)**	0.97 (0.83; 1.14)	0.94 (0.80; 1.11)	0.98 (0.84; 1.14)	0.98 (0.84; 1.14)	1.05 (0.89; 1.24)
Mild anemic	Mortality	All cause	**1.17 (1.04; 1.32)**	1.13 (0.99; 1.29)	**1.16 (1.03; 1.30)**	**1.15 (1.02; 1.29)**	**1.20 (1.06; 1.35)**	**1.33 (1.14; 1.56)**	**1.31 (1.09; 1.57)**	**1.31 (1.11; 1.53)**	**1.29 (1.10; 1.52)**	**1.32 (1.13; 1.55)**	1.02 (0.86; 1.21)	0.99 (0.83; 1.19)	1.02 (0.86; 1.21)	1.02 (0.86; 1.21)	1.06 (0.89; 1.26)
Anemic-Inflammation	Mortality	All cause	**1.19 (1.06; 1.33)**	**1.15 (1.01; 1.29)**	**1.18 (1.05; 1.32)**	**1.17 (1.05; 1.31)**	**1.21 (1.09; 1.36)**	**1.40 (1.20; 1.63)**	**1.39 (1.17; 1.65)**	**1.37 (1.18; 1.61)**	**1.36 (1.16; 1.59)**	**1.39 (1.19; 1.63)**	1.01 (0.86; 1.19)	0.98 (0.83; 1.16)	1.01 (0.86; 1.19)	1.01 (0.86; 1.19)	1.05 (0.89; 1.24)

*Model 1: adjusted by BMI and Physical activity; model 2: additionally adjusted by health at baseline and family history of MI/CVA; model 3: additionally adjusted by eGFR; model 4: additionally adjusted by smoking status, blood pressure and LDL-levels.

4. Discussion

By analyzing this large population-based study, which followed a middle-aged cohort of healthy individuals over a 21 year-period, we can conclude the following:

1 In men, the overall survival probability of anemic study participants is impaired, especially with rising age.
2 MI/CV incidence rates in men are influenced by abnormal HGB levels at baseline. However, associated mortality does not differ.
3 A change in HGB levels over time, i.e. from the first to the second health check up, is associated with excessive all-cause mortality in the overall study cohort, although it does not show a sex-specific correlation.

Prior studies in high risk cohorts, who are already diagnosed with CV disease, or present with many CV risk factors at baseline, suggest that lower HGB or lower hematocrit levels can be a risk factor for CV events and are associated impaired clinical outcomes [20,21].

Several population based studies [6–9] already tried to link hemoglobin and/or hematocrit levels to future health events in healthy adults and, considering the pathophysiological background, to the incidence of cardiovascular events. The results of these studies are rather opaque and do not necessarily point in the same direction. The Atherosclerosis Risk in Communities (ARIC; NCT00005131) study could show that the presence of anemia was independently associated with an increased risk of future CV disease events in the overall cohort [6]. The Cardiovascular Health Study (NCT00149435) investigated the impact of HGB concentrations on mortality in an elderly US-based population. The investigators found that lower and higher HGB levels and anemia, defined by the WHO criteria, were independently associated with increased all-cause mortality [22]. The Japanese Hisayama study focused on the association of elevated and decreased hematocrit levels and could show that the influences varies among different subtypes of CV disease [7]. Sex-specific differences became apparent in an analysis of the Framingham study (NCT00062777): women had a U-shaped relationship between hematocrit values and CV disease events, whereas hematocrit levels in younger men were linked to the occurrence of CV disease, including myocardial infarction and stroke [9]. Only one other study tried to identify the effect of hemoglobin kinetics in the general population and found that an increase or decrease of HGB levels outside the norm was associated with increased all-cause mortality [8]. The current study adds additional sex-specific insights and supports the idea that gender-differences play an important role in disease development, progression and treatment. Specifically focusing on cardiovascular events, this study could show that MI/CV incidence rates in men, especially in the elderly population, are influenced by abnormal HGB levels at baseline, although the associated mortality did not differ as compared to women. The change in HGB levels over time is a less explored topic in the general population. Only Lee et al. [8] investigated HGB change in a population of 292.194 healthy Koreans, aged 40 years or older. The study came to the conclusion that deviating HGB levels from the norm are associated with increased all-cause mortality in both sexes which could be confirmed by this study in a Caucasian population with a longer follow-up duration and various sex-specific models, taking several cardiovascular risk factors into account.

As already elucidated in the introduction, there are several potential reasons and pathophysiological explanations, why low and high HGB levels may be associated with mortality and the incidence of CV events in otherwise healthy individuals. Particularly interesting and noteworthy was also that not only anemic, but also mild anemic men and men suffering from anemia of inflammation had a higher risk of mortality, whilst none of the anemia forms were associated with a worse overall outcome in women. The observed sex-specific effects of abnormal hemoglobin levels on CV events and death in this observational study are not unequally explainable. Men and women have different HGB levels in health, which might be directly linked to the effect of sex hormones on erythropoiesis [23]- In our study, men showed a J-shaped association of HGB levels and the incidence for MI and other CV events after adjustment for other CV risk factors. Higher HGB levels (even within the normal range) are associated with the risk of thrombosis [14]. Thrombosis through higher HBG levels, and vasoconstriction mediated through androgens may be an explanatory theory why the male population suffers from a higher risk for CV events in general, although this remains speculative. However, sex-specific differences in this study may partially be explained by participant bias. Only clinically overt CV events were documented. Women seek medical attention less frequently than men and CV symptoms are often misdiagnosed [24,25]. This might partially explain, why none of the CV effects of anemia in men were also observed in women. In terms of the increased all-cause mortality in anemic men, this argument can not serve as a valid explanation. Death is an objective and definitive outcome which is not linked to clinical presentation and the interpretation of symptoms.

5. Conclusions

The influence of hemoglobin levels on future health events is sex-dependent: in healthy men, anemia decreases the overall survival probability, especially in the elderly population. After adjusting for several CV risk factors, low and especially high hemoglobin levels were associated with an increased incidence of MI/CV events. None of these effects were observed in women.

6. Limitations

There are several limitations to our analysis. Due to the observational nature of this investigation, we are not able to adjust for potentially not measured confounding factors. HGB analysis was performed according to local laboratory standards. However, we do not know in detail which tests where used. Since questionnaires were administered repetitively to participants, the Hawthorne effect could affect results, especially of the sequential modeling analysis. Multivariate analysis did not account for several relevant factors which might also be associated with the development of anemia and CV events such as the nutritional status and/or the socioeconomic status.

Funding

The EPIC—Norfolk study (DOI 10.22025/2019.10.105.00004) has received funding from the Medical Research Council (MR/N003284/1 MC-UU_12015/1 and MC_UU_00006/1) and Cancer Research UK (C864/A14136). The genetics work in the EPIC—Norfolk study was funded by the Medical Research Council (MC_PC_13048). We are grateful to all the participants who have been part of the project and to the many members of the study teams at the University of Cambridge who have enabled this research. CJ and MK were supported by the German Research Foundation (DFG, CRC 1116). Funding was also granted by the Forschungskommission of the Medical Faculty of the Heinrich-Heine-University Düsseldorf to PW for a Clinician Scientist Track.

Data availability

The data underlying this article were provided by the EPIC—Norfolk study consortium. Data will be shared on request to the corresponding author after approval by the EPIC—Norfolk study consortium.

Declaration of Competing Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

[1] WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva: World Health Organization; 2011.
[2] Stevens GA, Finucane MM, De-Regil LM, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. Lancet Glob Health 2013;1:e16–25.
[3] Stauder R, Valent P, Theurl I. Anemia at older age: etiologies, clinical implications, and management. Blood 2018;131:505–14.
[4] Anand IS, Gupta P. Anemia and iron deficiency in heart failure: current concepts and emerging therapies. Circulation 2018;138:80–98.
[5] Brener SJ, Mehran R, Dangas GD, et al. Relation of baseline hemoglobin levels and adverse events in patients with acute coronary syndromes (From the acute catheterization and urgent intervention triage strategY and Harmonizing Outcomes with RevasculariZatiON and stents in acute myocardial infarction trials). Am J Cardiol 2017;119:1710–6.
[6] 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.
[7] Gotoh S, Hata J, Ninomiya T, et al. Hematocrit and the risk of cardiovascular disease in a Japanese community: the Hisayama Study. Atherosclerosis 2015;242:199–204.
[8] Lee G, Choi S, Kim K, et al. Association of hemoglobin concentration and its change with cardiovascular and all-cause mortality. J Am Heart Assoc 2018;7.
[9] 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;127:674–82.
[10] Varat MA, Adolph RJ, Fowler NO. Cardiovascular effects of anemia. Am Heart J 1972;83:415–26.
[11] Gerry JL, Baird MG, Fortuin NJ. Evaluation of left ventricular function in patients with sickle cell anemia. Am J Med 1976;60:968–72.
[12] Anand IS, Chandrashekhar Y, Ferrari R, Poole-Wilson PA, Harris PC. Pathogenesis of oedema in chronic severe anaemia: studies of body water and sodium, renal function, haemodynamic variables, and plasma hormones. Br Heart J 1993;70:357–62.
[13] 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(3):14–8. Suppl.
[14] Danesh J, Collins R, Peto R, Lowe GD. Haematocrit, viscosity, erythrocyte sedimentation rate: meta-analyses of prospective studies of coronary heart disease. Eur Heart J 2000;21:515–20.
[15] Lowe GD, Forbes CD. Platelet aggregation, haematocrit, and fibrinogen. Lancet 1985;1:395–6.
[16] Pratico D, Pasin M, Barry OP, et al. Iron-dependent human platelet activation and hydroxyl radical formation: involvement of protein kinase C. Circulation 1999;99:3118–24.
[17] Day N, Oakes S, Luben R, Khaw KT, Bingham S, Welch A, Wareham N. EPIC-Norfolk: study design and characteristics of the cohort. European prospective investigation of cancer. Br J Cancer 1999;80(1):95–103. Suppl.
[18] Sinha S, Myint PK, Luben RN, Khaw KT. Accuracy of death certification and hospital record linkage for identification of incident stroke. BMC Med Res Methodol 2008;8:74.
[19] Pana TA, Luben RN, Mamas MA, Potter JF, Wareham NJ, Khaw KT, et al. Long Term Prognostic Impact of Sex-specific Longitudinal Changes in Blood Pressure. The EPIC-Norfolk Prospective Population Cohort Study. Eur J Prev Cardiol 2022;29(1):180–91.
[20] Barlas RS, Honney K, Loke YK, et al. Impact of hemoglobin levels and anemia on mortality in acute stroke: analysis of UK regional registry data, systematic review, and meta-analysis. J Am Heart Assoc 2016;5(8):e003019.
[21] Sabatine MS, Morrow DA, Giugliano RP, et al. Association of hemoglobin levels with clinical outcomes in acute coronary syndromes. Circulation 2005;111:2042–9.
[22] Zakai NA, Katz R, Hirsch C, Shlipak MG, Chaves PH, Newman AB, Cushman M. A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the cardiovascular health study. Arch Intern Med 2005;165:2214–20.
[23] Murphy WG. The sex difference in haemoglobin levels in adults - mechanisms, causes, and consequences. Blood Rev 2014;28:41–7.
[24] Martínez-Nadal G, Miró Ò, Matas A, et al. An analysis based on sex&gender in the chest pain unit of an emergency department during the last 12 years. Eur Heart J Acute Cardiovasc Care 2021;10:zuab020.122.
[25] Clerc Liaudat C, Vaucher P, De Francesco T, et al. Sex/gender bias in the management of chest pain in ambulatory care. Womens Health 2018;14:1745506518805641 *(Lond)*.