

Association of anemia with health-related quality of life and survival: a large population-based cohort study

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Supplemental data

Lifelines

Lifelines employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics (lifelines.net)¹. The inclusion route and exclusion criteria are shown in Supplemental figure 1. All participants were between 18 and 93 years old at the time of enrolment. The Lifelines Cohort Study states that the study complies with the Declaration of Helsinki.

Clinical examination

All subjects completed a self-administered questionnaire on medical history, past and current diseases, use of medication, and health behavior at home. Medication use was verified by a certified research assistant, and scored by ATC code. The number of different medications used by a participant was considered as a proxy for multi-morbidity². Smoking status was defined as non-smoker, former smoker and current smoker (including the use of cigarettes, cigarillos, cigars and pipe tobacco). Body weight was measured without shoes to the nearest 0.1 kg. Height and waist and hip circumference were measured to the nearest 0.5 cm. Body mass index (BMI) was calculated by dividing weight in kilograms by the squared height in meters (kg/m²). Heart rate and systolic and diastolic blood pressure were measured every minute for a period of 10 minutes using a DINAMAP Monitor. The size of the cuff was chosen according to the arm circumference. The average of the last three measurements was reported.

Biochemical measurements

Blood samples were collected in the morning after an overnight fast between 2006 and 2013. The blood samples were placed at 4°C and transported from the Lifelines research site to the Lifelines laboratory, under tightly controlled and continuously monitored conditions. From the Lifelines laboratory, part of the samples were directly transferred to the central laboratory of the University Medical Center Groningen, to perform routine clinical chemistry assays on fresh samples. The other part of the samples remained at the Lifelines laboratory. These samples were stored in two-dimensional barcoded aliquots at -80 °C, to allow for future measurements³. All measurements, except analysis of hepcidin were performed in the clinical chemistry laboratory of the University Medical Center Groningen. Hemoglobin, total leucocytes and thrombocytes were measured using routine procedures on a XE2100-system (Sysmex, Japan). HbA1c was measured using a turbidimetric inhibition immunoassay on a Cobas Integra 800 CTS analyzer (Roche Diagnostics Nederland BV, Almere, Netherlands). Fasting blood glucose was measured using a hexokinase method, and hs-CRP was analyzed using nephelometry (BN II system; Siemens, Marburg, Germany). TSH, fT4, and fT3 were assayed by electro-chemiluminescent immunoassay on the Roche Modular E170 Analyzer using kits provided by the manufacturer (Roche, Basel, Switzerland). Serum creatinine was measured on a Roche Modular P chemistry analyzer (Roche, Basel Switzerland). Alanine aminotransferase (ALT) was measured with pyridoxal phosphate activation on a Roche Modular Platform. Serum vitamin B12 and folate concentration were measured by electrochemiluminescence immunoassay (Roche Modular, Mannheim, Germany). Serum methylmalonic acid was measured by liquid chromatography–mass spectrometry (Spark Holland Symbiosis combined with Waters Quattro Premier XE). Hepcidin measurements were performed in Nijmegen (Hepcidinanalysis.com, Nijmegen, The Netherlands) using a competitive ELISA assay^{4,5}.

HRQoL

The RAND-36 includes the same items as the 36-Item Short Form Health Survey v1.0, except the scoring for the two subscales of bodily pain and general health are slightly different. This generic questionnaire consists of 36 items in eight subscales: physical functioning (ten items), physical role functioning (four items), bodily pain (two items), general health (five items), vitality (four items), social functioning (two items), emotional role functioning (three items), and mental health (five items). A higher score reflects a better health status and well-being.

Different classification of anemia

Criteria to classify anemia were based on Guralnik et al. and Weiss et al.^{6,7} Iron deficiency, was considered present if the participant had 2 or 3 of the following criteria: serum ferritin concentration < 30 (men) / 15 (women) µg/L or transferrin saturation rate < 15% or hepcidin < 0.5 nmol/L. Transferrin saturation was calculated by dividing serum iron by total iron-binding capacity ((transferrin (g/L) x 25). Folate deficiency was defined as folate serum level < 9.8 nmol/L. Vitamin B12 deficiency was defined as serum methylmalonic acid concentration > 340 nmol/L. If there was no evidence of nutrient deficiency, subjects with anemia were evaluated for other causes. Subjects were classified as having anemia related to chronic renal disease if the estimated glomerular filtration rate was < 30 mL/min. The CDK-EPI formula was used to calculate the eGFR. Anemia of chronic inflammation (ACI) was defined as serum iron concentration (< 10 µmol/L) without evidence of iron deficiency or as serum ferritin concentration > 400 (men) / 130 (women) µg/L. If subjects with anemia could not be classified into any of these categories, they were considered, by exclusion, to have unexplained anemia (UA).

Data description and statistical analysis

All analyses were conducted using IBM SPSS Statistics for Windows v22 (IBM, Armonk, NY). Data are presented as mean (standard deviation), or median (interquartile range). Between-group differences were evaluated using one way ANOVA, Kruskal-Wallis test, or Chi-square test, as appropriate. A multivariate logistic-regression model was used to determine the independent effect of anemia on the odds and 95% confidence intervals of having a lower score than the (sex- and age- specific) cut-off per HRQoL subscale. The model was adjusted for variables known to influence health-related quality of life, including body mass index (\leq / $>$ 30 kg/m²)⁸, smoking status (current/former/non-smoker)⁹, polypharmacy (\geq 4 different medications)¹⁰, educational level (elementary/ lower secondary / upper secondary/ tertiary)¹¹, and living situation (independently / dependently). The Hosmer- Lemeshow test was used to check the goodness-of-fit of the model. Logistic regression was also used to determine the optimal definition of anemia in the perspective of total HRQoL score. Survival was analyzed by Kaplan-Meier statistics and compared between the groups by the Log-Rank test. To adjust for multiple comparisons, a p-value of <.01 was considered statistically significant. All p-values were two-sided.

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Supplemental table 1: Classification of anemia

	Men (N=219)	Women (N=278)
Nutrient deficiency anemia		
Iron deficiency	53	63
Folate deficiency	1	5
Vitamin B12 deficiency	20	17
Combined	8	7
Anemia of chronic inflammation	44	37
Chronic kidney disease	6	1
Unexplained anemia	93	149

Data given as n of participants per type of anemia.

Classification of anemia was performed in 497 of 516 anemic individuals.

Supplemental table 2: Percentage of individuals older than 60 years with anemia with HRQoL below the sex- and age-specific cut-off value for the different HRQoL domains as function of their type of anemia – men

	General population older than 60 years (N=8451)	Nutrient deficiency anemia (N=82)	Anemia of chronic inflammation (N=44)	Unexplained anemia (N=93)
Physical functioning	2317 (27.4)	32 (39.0)	23 (52.3)* p < .001	3 (35.5)
Social functioning	2919 (34.5)	32 (39.0)	25 (56.8)* p = .002	36 (38.7)
Physical role functioning	1594 (18.9)	23 (28.0)	25 (56.8)* p < .001	23 (24.7)
Emotional role functioning	692 (8.2)	9 (11.0)	9 (20.5)* p < .003	8 (8.6)
Mental health	2571 (30.4)	23 (28.0)	18 (40.9)	28 (30.1)
Vitality	2140 (25.3)	19 (23.2)	24 (54.5)* p < .001	28 (30.1)
Bodily pain	2437 (28.8)	28 (34.1)	23 (52.3)* p = .001	35 (37.6)
General health	2209 (26.1)	29 (35.4)	20 (45.5)* p = .004	30 (32.3)

Data are given as n (%) below the sex- and age-specific cut-off value for the different HRQoL domains.

* p-value <.01 between individuals from the non-anemic individuals from the general population and the type of anemia.

Supplemental table 3: Percentage of individuals older than 60 years with anemia with HRQoL below the sex- and age-specific cut-off value for the different HRQoL domains as function of their type of anemia – women

	General population older than 60 years (N=9700)	Nutrient deficiency anemia (N=92)	Anemia of chronic inflammation (N=37)	Unexplained anemia (N=149)
Physical functioning	2552 (26.3)	35 (38.0)	17 (45.9)* p = .007	45 (30.2)
Social functioning	2563 (26.4)	21 (22.8)	16 (43.2)	49 (32.9)
Physical role functioning	2226 (23.0)	26 (28.3)	16 (43.2)* p = .003	40 (26.8)
Emotional role functioning	1244 (12.8)	11 (12.0)	8 (21.6)	22 (14.8)
Mental health	2645 (27.3)	19 (20.7)	8 (21.6)	44 (29.5)
Vitality	2680 (27.6)	30 (32.6)	15 (38.5)	47 (31.5)
Bodily pain	3181 (32.8)	32 (34.8)	17 (45.9)	60 (40.3)
General health	2895 (29.8)	31 (33.7)	20 (54.1)* p = .001	49 (32.9)

Data are given as n (%) below the sex- and age-specific cut-off value for the different HRQoL domains.

* p-value <.01 between individuals from the non-anemic individuals from the general population and the type of anemia.

Supplemental table 4: Risk of having a lower score than the (age- and sex- specific) 25th percentile cut-off due to anemia per HRQoL domain older than 60 years for men and women

Physical functioning	Nutritional deficiency anemia	Men	1.28 (0.70 – 2.33)
		Women	0.84 (0.47 – 1.56)
	Anemia of chronic inflammation	Men	2.51 (1.10 – 5.79)*
		Women	4.16 (1.71 – 10.2)** p=.002
	Unexplained anemia	Men	0.86 (0.52 – 1.44)
		Women	1.13 (0.70 – 1.80)
Social functioning	Nutritional deficiency anemia	Men	1.01 (0.57 - 1.78)
		Women	0.76 (0.41 – 1.41)
	Anemia of chronic inflammation	Men	2.07 (0.93 – 4.57)
		Women	2.80 (1.22 – 6.47)* p=.02
	Unexplained anemia	Men	0.88 (0.55 - 1.43)
		Women	1.21 (0.78 – 1.87)
Physical role functioning	Nutritional deficiency anemia	Men	1.26 (0.66 – 2.37)
		Women	1.13 (0.62 – 2.05)
	Anemia of chronic inflammation	Men	5.05 (2.22- 11.51)**
		Women	p < .001 2.80 (1.20 – 6.52)* p=0.02
	Unexplained anemia	Men	0.93 (0.54 - 1.60)
		Women	1.07 (0.67 – 1.72)
Emotional role functioning	Nutritional deficiency anemia	Men	0.96 (0.38 – 2.47)
		Women	0.94 (0.45 – 1.96)
	Anemia of chronic inflammation	Men	3.14 (1.29 – 7.68)**
		Women	p = .006 2.10 (0.81 – 5.43)
	Unexplained anemia	Men	0.75 (0.32 – 1.75)
		Women	1.20 (0.69 – 2.08)
Vitality	Nutritional deficiency anemia	Men	0.91 (0.48 - 1.71)
		Women	0.96 (0.54 – 1.74)
	Anemia of chronic inflammation	Men	3.81 (1.69 – 8.61)**
		Women	p < .001 2.16 (0.93 – 5.04)
	Unexplained anemia	Men	1.15 (0.70 - 1.88)
		Women	1.08 (0.69 – 1.69)
Bodily pain	Nutritional deficiency anemia	Men	1.06 (0.65 - 1.72)
		Women	1.01 (0.57 – 1.80)
	Anemia of chronic inflammation	Men	2.24 (1.01 – 4.95)*
		Women	p = .05 1.60 (0.69 – 3.72)
	Unexplained anemia	Men	1.06 (0.65 – 1.72)
		Women	1.20 (0.78 – 3.72)
General health	Nutritional deficiency anemia	Men	1.37 (0.77 – 2.46)
		Women	0.58 (0.32 – 1.08)
	Anemia of chronic inflammation	Men	1.64 (0.74 – 3.68)
		Women	4.04 (1.67 – 9.88)** p<.002
	Unexplained anemia	Men	0.93 (0.57 – 1.59)

		Women	1.02 (0.66 – 1.59)
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Adjusted for body mass index, smoking status, polypharmacy, educational level and living situation.

Data are shown as OR and 95% CI.

**** p-value < .01** *** p-value < .05**

Supplemental table 5: Percentage of individuals older than 60 years with anemia with HRQoL below the sex- and age-specific cut-off value for the different HRQoL domains as function of their type of anemia (using different criteria).

	General population older than 60 years (N=18151)	Nutrient deficiency anemia (N=167)	Anemia of chronic inflammation (N=129)	Unexplained anemia (N=201)
Physical functioning	4869 (26.8)	65 (38.9)* p = .001	48 (37.2)* p = .008	72 (35.8)* p = .004
Social functioning	5482 (30.2)	52 (31.1)	54 (41.9)* p = .004	73 (36.3)
Physical role functioning	3820 (21.0)	47 (28.1)	51 (39.5)* p < .001	55 (27.4)
Emotional role functioning	1936 (10.7)	17 (10.2)	17 (13.2)	33 (16.4)
Mental health	5216 (28.7)	41 (24.6)	36 (27.9)	63 (31.3)
Vitality	4820 (26.5)	48 (28.6)	49 (37.7)* p = .004	66 (32.8)
Bodily pain	5618 (30.9)	55 (32.9)	57 (44.2)* p = .001	83 (41.3)* p = .002
General health	5104 (28.1)	59 (35.3)	50 (38.8)* p = .009	70 (34.8)

Data are given as n (%) below the sex- and age-specific cut-off value for the different HRQoL domains.

* p-value <.01 between individuals from the non-anemic individuals from the general population and the type of anemia.

Supplemental table 6: Risk of having a lower score than the (age- and sex- specific) 25th percentile cut-off due to anemia per HRQoL domain older than 60 years (using different criteria).

Physical functioning	Nutritional deficiency anemia	1.14 (0.74 – 1.76)
	Anemia of chronic inflammation	1.79 (1.12 – 2.79)* p = .02
	Unexplained anemia	1.01 (0.70 – 1.47)
Social functioning	Nutritional deficiency anemia	0.96 (0.63 - 1.45)
	Anemia of chronic inflammation	1.48 (0.96 – 2.29)
	Unexplained anemia	1.06 (0.74 - 1.52)
Physical role functioning	Nutritional deficiency anemia	1.19 (0.76 – 1.84)
	Anemia of chronic inflammation	2.24 (1.44- 3.51)** p < .001
	Unexplained anemia	1.01 (0.68 - 1.50)
Emotional role functioning	Nutritional deficiency anemia	0.82 (0.45 – 1.52)
	Anemia of chronic inflammation	1.48 (0.84 – 2.61)
	Unexplained anemia	1.28 (0.80 – 2.04)
Vitality	Nutritional deficiency anemia	0.96 (0.62 - 1.48)
	Anemia of chronic inflammation	1.67 (1.07 – 2.60)* p = .02
	Unexplained anemia	1.20 (0.84 - 1.73)
Bodily pain	Nutritional deficiency anemia	0.87 (0.57 - 1.32)
	Anemia of chronic inflammation	1.68 (1.09 – 2.60)* p = .02
	Unexplained anemia	1.13 (0.80 – 1.61)
General health	Nutritional deficiency anemia	0.95 (0.62 - 1.46)
	Anemia of chronic inflammation	1.77 (1.14 – 2.76)* p = .01
	Unexplained anemia	0.90 (0.62 – 1.30)

Adjusted for body mass index, smoking status, polypharmacy, educational level and living situation.

Data are shown as OR and 95% CI.

** p-value < .01

* p-value < .05

Supplemental figure 1: Inclusion route and exclusion criteria Lifelines cohort¹

Supplemental figure 2: Distribution of hemoglobin concentration

eFigure 2A and 2C show the distribution of the hemoglobin concentration according to age for both men and women. eFigure 2B and 2D show the frequency of the hemoglobin concentrations in individuals younger and older than 60 years for both men (mean 9.42 and 9.27 mmol/L) and women (mean 8.30 and 8.49 mmol/L).

Supplemental figure 3: Mean total HRQoL score and percentage below sex- and age-specific cut-off value

An asterisk indicates a significantly (p-value < .01) lower mean score or larger percentage below the sex- and age-specific cut-off value in anemic individuals compared with non-anemic individuals.

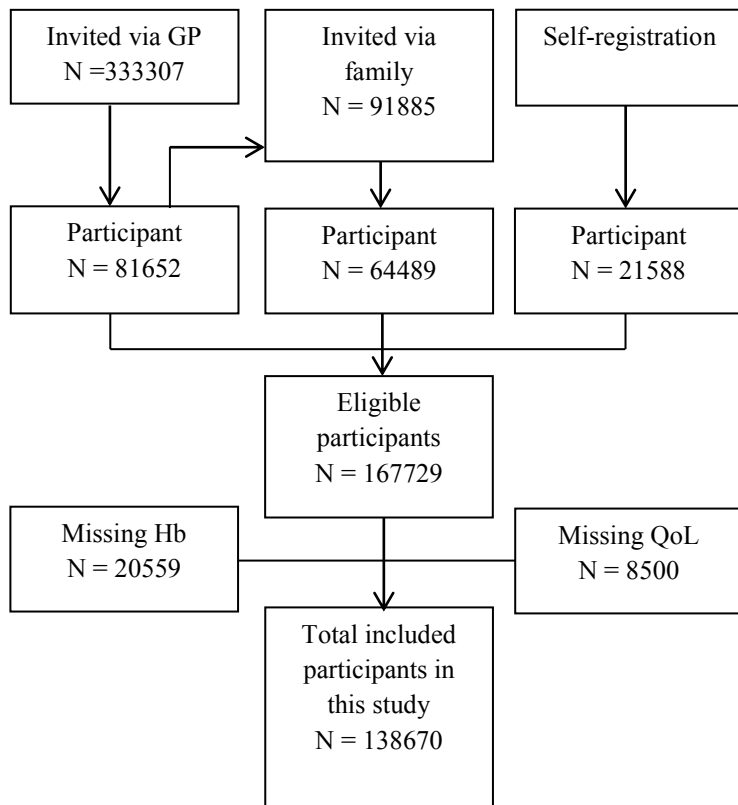
Supplemental figure 4: Mean score of the different domains of the RAND-36 (-item health survey)

An asterisk indicates a significantly (p-value < .01) lower mean HRQoL score in anemic individuals compared with non-anemic individuals.

PF; physical functioning , SF; social functioning, RF; physical role functioning , RE; emotional role functioning
MH; mental health, VT; vitality, BP; bodily pain , GH; general health.

Supplemental figure 5: Median hemoglobin values for men and women as function of age

Supplemental figure 1: Inclusion route and exclusion criteria Lifelines cohort (1)

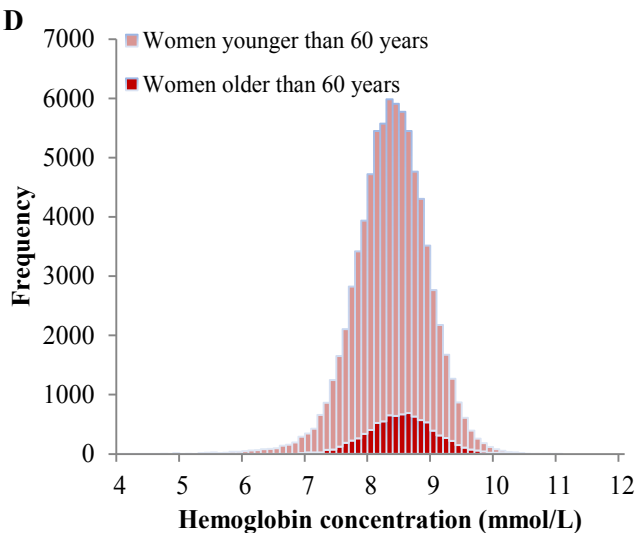
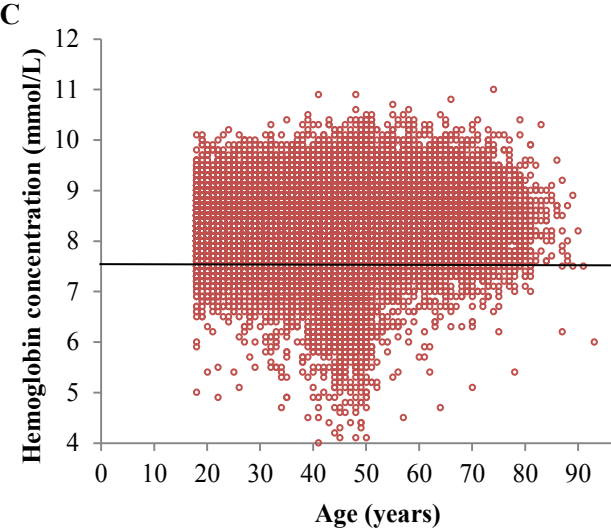
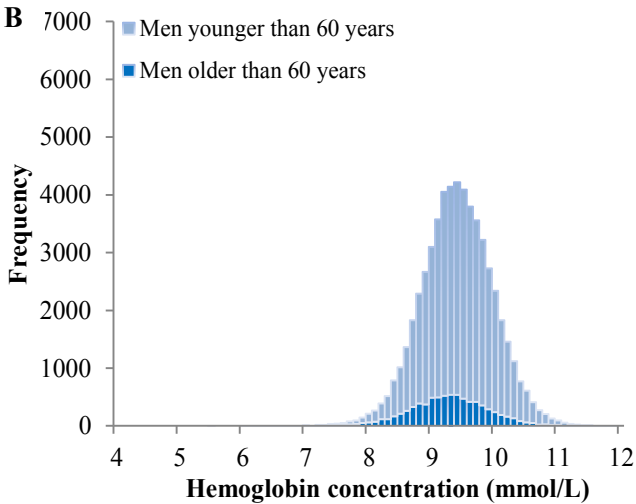
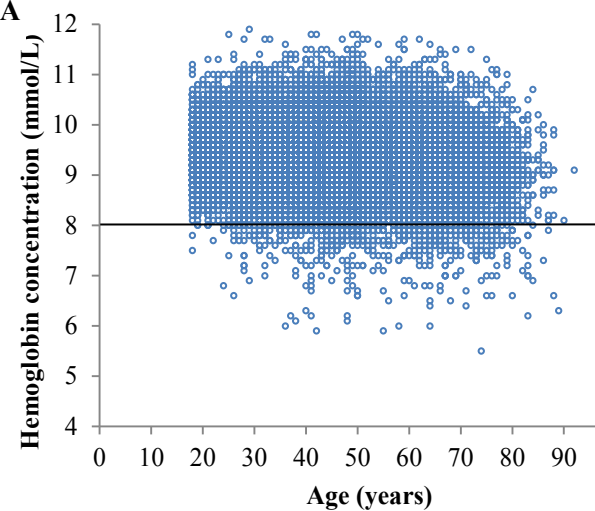


Exclusion criteria of the Lifelines cohort:

- Severe psychiatric or physical illness;
- Limited life expectancy (< 5 years);
- Insufficient knowledge of the Dutch language to complete a Dutch questionnaire.

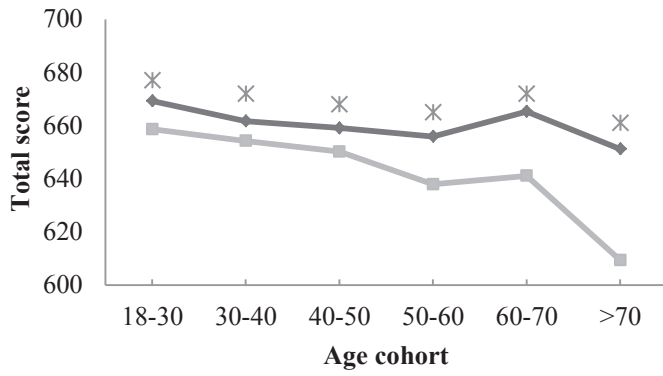
1. Scholtens S, Smidt N, Swertz MA, et al. Cohort profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol.* 2015;44(4):1172-1180.

Supplemental figure 2A-2D: Distribution of hemoglobin concentration

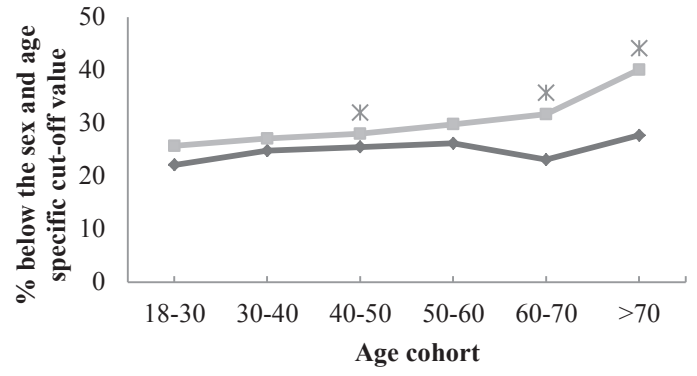


Supplemental figure 3: Mean total HRQoL score and percentage below sex- and age-specific cut-off value

Total score HRQoL - Mean



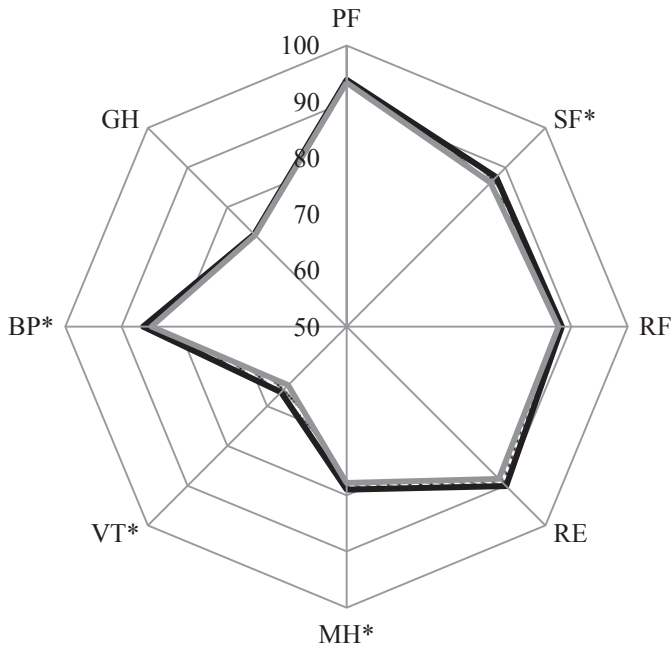
Total score HRQoL - %



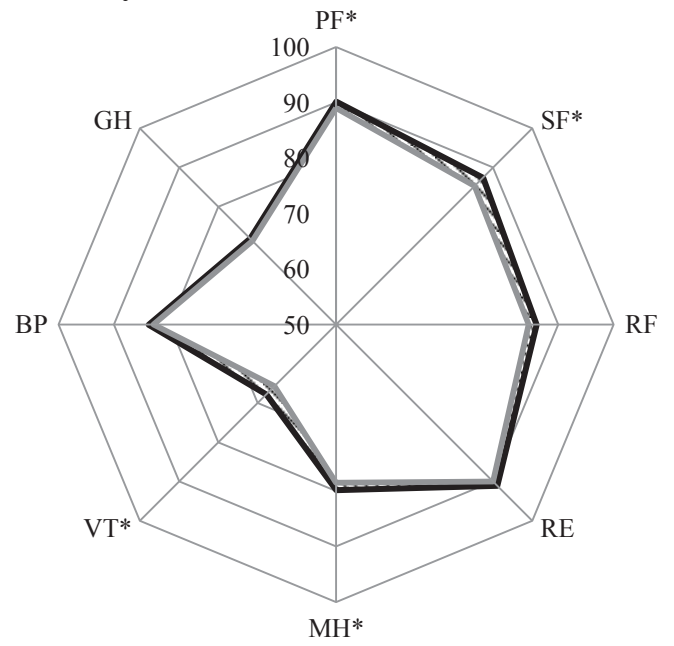
◆ Non-anemic individuals ■ Anemic individuals

Supplemental figure 4: Mean score of the different domains of the RAND-36 (-item health survey)

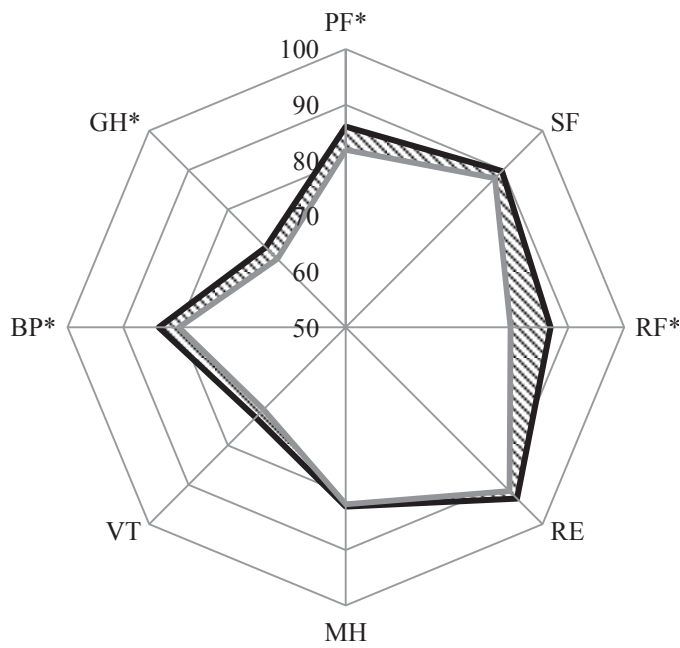
A 18 - 40 years



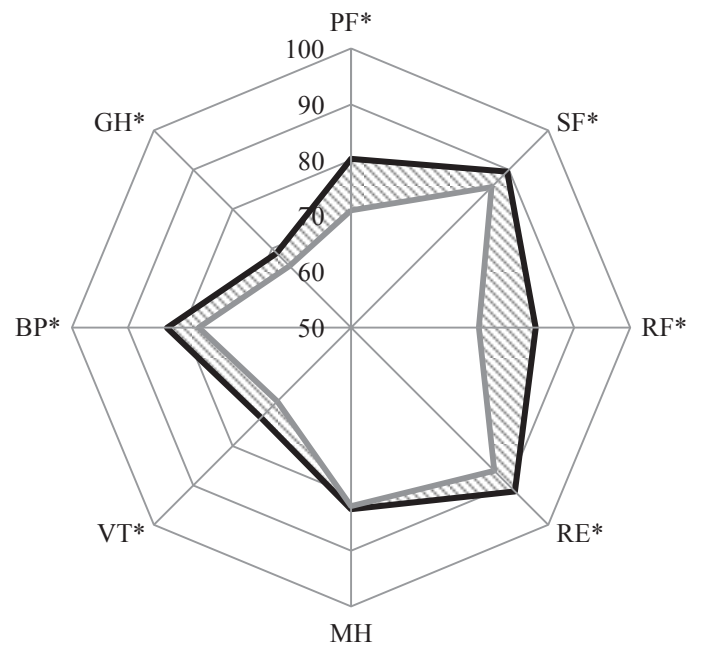
B 41 - 60 years



C 61 - 70 years



D >70 years



— Non-anemic individuals — Anemic individuals

Supplemental figure 5: Median hemoglobin values for men and women as function of age

