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ORIGINAL RESEARCH ARTICLE

Association of iron deficiency with incident cardiovascular diseases and mortality in the general population

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Abstract

Aims Although absolute (AID) and functional iron deficiency (FID) are known risk factors for patients with cardiovascular (CV) disease, their relevance for the general population is unknown. The aim was to assess the association between AID/FID with incident CV disease and mortality in the general population.

Methods and results In 12 164 individuals from three European population-based cohorts, AID was defined as ferritin $<$ 100 μg/L or as ferritin $<$ 30 μg/L (severe AID), and FID was defined as ferritin $<$ 100 μg/L or ferritin 100–299 μg/L and transferrin saturation $<$ 20%. The association between iron deficiency and incident coronary heart disease (CHD), CV mortality, and all-cause mortality was evaluated by Cox regression models. Population attributable fraction (PAF) was estimated. Median age was 59 (45–68) years; 45.2% were male. AID, severe AID, and FID were prevalent in 60.0%, 16.4%, and 64.3% of individuals. AID was associated with CHD [hazard ratio (HR) 1.20, 95% confidence interval (CI) 1.04–1.39, P = 0.01], but not with mortality. Severe AID was associated with all-cause mortality (HR 1.28, 95% CI 1.12–1.46, $P < 0.01$), but not with CV mortality/CHD. FID was associated with CHD (HR 1.24, 95% CI 1.07–1.43, $P < 0.01$), CV mortality (HR 1.26, 95% CI 1.03–1.54, P = 0.03), and all-cause mortality (HR 1.12, 95% CI 1.01–1.24, P = 0.03). Overall, 5.4% of all deaths, 11.7% of all CV deaths, and 10.7% of CHD were attributable to FID.

Conclusions In the general population, FID was highly prevalent, was associated with incident CHD, CV death, and all-cause death, and had the highest PAF for these events, whereas AID was only associated with CHD and severe AID only with all-cause mortality. This indicates that FID is a relevant risk factor for CV diseases in the general population.

Keywords Iron deficiency; Risk factor; General population; Cardiovascular; Mortality

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Introduction

Iron is essential for the homeostasis of the human body. It plays a central role in oxygen transport and utilization as well as in mitochondrial function.[1] Historically, iron status has been assessed by measuring ferritin serum levels, and patients with low ferritin serum levels have been diagnosed with absolute iron deficiency (AID) or severe AID. In such

patients, anaemia and skeletal muscle dysfunction is most likely present.[2]

However, ferritin alone might not suffice to provide an accurate and reliable assessment of iron status, as it solely reflects stored iron. Transferrin saturation (TSAT) should be additionally measured to account for utilized iron. Measurement of both markers provides a more accurate description of iron status and can identify patients with a functional iron deficiency (FID).[3]

Recently, several cardiovascular studies have indicated an association of AID and FID with morbidity and mortality in patients with cardiovascular diseases (CVDs) such as coronary heart disease (CHD), acute myocardial infarction, and heart failure.[4–7] These findings have led to the initiation of a randomized controlled trial of iron supplementation vs. placebo in patients with heart failure (FAIR-HF).[8] Here, iron supplementation showed a significant improvement in functional endpoints, an effect that was independent of the presence of anaemia.[9] Based on this trial, a second randomized controlled trial of iron supplementation in patients with heart failure has been launched (FAIR-HF II). This second trial will assess a mortality endpoint and is expected to be reported within the next years (NCT03036462). Both trials used an updated definition of FID, based on either low ferritin ($<$100 μg/L) or intermediate ferritin ($\geq$100 μg/L but $<$300 μg/L) with a low TSAT ($<$20%).

Based on the association of AID and FID with outcome in patients with CVD, it could be speculated that iron deficiency (ID) might also play an important role in the general population. However, previous analyses have studied ID mostly based on ferritin-only definitions in the general population (e.g. assessing low or very low levels of stored iron, AID, and severe AID) and failed to show a consistent association with distinct outcomes after adequate adjustment.[10,11] Assessing FID, and thereby assessing utilized iron as well as stored iron, provides a more comprehensive picture of the iron status and might therefore be a reliable risk marker for incident CVD and mortality, even at the general population level.

Hence, the aim of this study was to assess the association between AID/FID with incident CVD and mortality in the general population.

Methods

Study sample

This study is based on data of the MORGAM (Monica Risk, Genetics, Archiving and Monograph)/BiomarCaRE (Biomarker for Cardiovascular Risk Assessment in Europe) consortium, providing harmonized risk factors and biomarker data and endpoints from large-scale European population-based cohort studies.[12] Ferritin and TSAT at baseline were available in 12 146 individuals from three cohort studies (KORA, Northern Sweden, and Tromsø). All participating cohort studies were approved by local ethics committees, and informed consent was obtained from each participant. Details on the enrolment and follow-up procedures for each cohort study are provided in the Supporting Information, *Appendix S1*.

Definition of iron deficiency and measurement of iron markers

There is no general consensus on the definition of severe AID. An early study by Mast *et al*. suggests that a ferritin cut-off of $\leq$30 μg/L has an optimal specificity/sensitivity to diagnose severe AID and has therefore been widely adopted.[13] However, there is also reasonable evidence to use an even lower ferritin cut-off ($\leq$15 μg/L) in individuals aged 20+ years as suggested by the *World Health Organization* (WHO), although this should be corrected in the presence of infection/inflammation (even if subclinical).[14,15] For clarity of message, we opted to use one cut-off to define severe AID in this study. We hence selected the ferritin cut-off suggested by Mast *et al*. ($\leq$30 μg/L),[13] which is not only a well-established cut-off in this field but also seems more likely to account for the higher age (and therefore slightly higher levels of inflammation) of our study population (e.g. a lower ferritin cut-off might have led to more false negatives).

Several trials have indicated that AID and FID are associated with morbidity and mortality in patients with CVD. Furthermore, the FAIR-HF trial has indicated a substantial morbidity benefit of correcting FID/AID in heart failure patients (a finding that is currently further evaluating regarding a potential mortality benefit in the FAIR-HF II trial).[8] Therefore, we used the same definition for AID and FID as the FAIR-HF trial, to test the hypothesis that such an association can also be observed in the general population.

Ultimately, AID was defined as ferritin $<$ 100 μg/L and severe AID as ferritin $<$ 30 μg/L. FID was defined as ferritin $<$ 100 μg/L or ferritin 100–299 μg/L and TSAT $<$ 20%.

In KORA, ferritin was measured by electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany), iron by colorimetry (Roche Diagnostics), and transferrin by immunonephelometry (Dade Behring). TSAT was calculated using the following formula: [Iron (μmol/L)/transferrin (g/L)] * 3.98.

In Northern Sweden, ferritin was measured by enzyme-linked immunosorbent assay with the antibody ILS/R070 (Axel Johnson, Stockholm, Sweden), iron by ferrozine method (Hitachi 717 analyser, Boehringer, Mannheim, Germany), and total iron binding capacity (TIBC) as unbound iron binding capacity (Hitachi 717 analyser) plus iron. TSAT was calculated using the following formula: [Iron (μmol/L)/TIBC] * 100.

In Tromsø, ferritin and transferrin were measured by turbidimetric assay and iron by ferrozine method (Hitachi 917 analyser, Boehringer). TSAT was calculated using the following formula: [Iron (μmol/L)/transferrin (g/L)] * 3.98.

All biomarkers were measured from serum.

Risk factors and follow-up

Risk factor information was collected at the baseline visit. The variables body mass index (BMI), systolic blood pressure (SBP), and total cholesterol were measured locally by routine methods similar to the WHO MONICA protocol (https://www.thl.fi/publications/monica/manual/index.htm). Information on diabetes, history of CVDs, and smoking was obtained by self-report. All data from the cohort studies were harmonized in the MORGAM project.[16] Data on missing values are displayed in the Supporting Information, *Appendix S1*.

High-sensitive C-reactive protein (hsCRP) was measured by immunonephelometry (BNII Analyser, Siemens, Germany) in KORA and by latex immunoassay (Abbott, Architect c8000) in Northern Sweden. In Tromsø, hsCRP was measured by a particle-enhanced immunoturbidimetric assay (Roche, Germany).

Incident CHD and incident stroke were adjudicated based on event registries, national hospital discharge registry data, or by self-report of the participants (with subsequent validation by medical records). All-cause and cardiovascular mortality data were derived from central death registries. Cardiovascular death was defined as coronary death validated by autopsy report, death certificates, and chart reviews (KORA) or fatal myocardial infarction and stroke identified via mortality register (Northern Sweden and Tromsø) (http://www.thl.fi/publications/morgam/cohorts/index.html). All endpoints were harmonized. The study period from baseline examination to the end of follow-up was between 1990 and 2008 for all cohorts (detailed information by study cohort is provided in the Supporting Information, *Appendix S1*).

Statistical analysis

Continuous variables are shown as median (Q1–Q3). For binary variables, absolute and relative frequencies are shown. The Wilcoxon rank-sum test (for continuous variables) or the χ^2 test (for categorical variables) was used for comparisons between groups.

Event rates were estimated using the Kaplan–Meier method. For the incident disease analyses, individuals with prevalent CHD or stroke were excluded. Multivariable-adjusted sex and centre stratified Cox regression analyses were performed to evaluate the association of AID, severe AID, and FID with incident CHD, incident stroke, all-cause mortality, and cardiovascular mortality. Adjustments were performed for age (time scale), sex (strata), smoking, total cholesterol, SBP, diabetes, BMI, hsCRP, and study centre (strata). A two-sided *P*-value of <0.05 was considered statistically significant.

For all endpoints 10 year population attributable fractions (PAFs) were calculated for AID, severe AID, and FID. PAF estimates the proportion of events in 10 years, which would have been avoided if all individuals had the risk of those without AID, severe AID, or FID at baseline. PAFs were calculated based on the equations of Laaksonen *et al*., which take into account the time-to-event nature of the data. For cardiovascular death or incident diseases, death from other causes was treated as competing risk and in that case the PAF computation was based on cause-specific Cox models. For all-cause mortality, a Cox model was used. All models were adjusted as described earlier. To estimate the baseline hazard function of each Cox model, a Weibull model was fitted. This model used age as the time scale and the linear predictor from the corresponding Cox model as the sole predictor. PAF confidence intervals (CIs) were computed by bootstrapping using 1000 samples.

All analyses were performed with R statistical software Version 3.6.3.

Results

Baseline characteristics and prevalence of iron deficiency

A total of 12 164 individuals were included in the analysis. The median age of the study population was 59 (45–68) years; 45.2% were male. Baseline characteristics are shown in *Tables 1–3*.

Among the 12 164 individuals, 7296 (60.0%) had ferritin $<$ 100 μg/L and fulfilled the criteria of AID, whereas 1989 (16.4%) had ferritin $<$ 30 μg/L and fulfilled the criteria of severe AID; and 7825 (64.3%) individuals had ferritin between 100 and 299 μg/L and TSAT $<$ 20% and fulfilled the criteria of FID (*Figure 1*).

Individuals with AID, severe AID, or FID were more likely to be female and were younger. Cardiovascular risk factors were heterogeneously distributed among groups: individuals with AID or FID were more likely to be smokers, whereas SBP, BMI, and total cholesterol were lower in these two groups and individuals with severe AID as compared with individuals without any form of ID. Individuals with severe AID were less likely to have had a history of myocardial infarction or stroke as compared with individuals without severe AID, although this was not observed in the other two groups (*Tables 1–3*).

Table 1 Baseline characteristics stratified by absolute iron deficiency

	All (N = 12 164)	No absolute iron deficiency (N = 4868)	Absolute iron deficiency (N = 7296)	P-value
Men, no. (%)	5493 (45.2)	3216 (66.1)	2277 (31.2)	<0.001
Age (years)	59 (45, 68)	60 (48, 68)	58 (43, 68)	<0.001
Daily smoker, no. (%)	2956 (24.4)	1071 (22.1)	1885 (26.0)	<0.001
Prevalent diabetes, no. (%)	537 (4.5)	297 (6.2)	240 (3.3)	<0.001
Hypertension, no. (%)	5508 (45.5)	2456 (50.7)	3052 (42.1)	<0.001
BMI (kg/m^2)	26.1 (23.7, 29.1)	27.1 (24.8, 30.1)	25.4 (23.0, 28.4)	<0.001
Systolic blood pressure (mmHg)	130 (117, 146)	132 (120, 147)	129 (115, 146)	<0.001
Total cholesterol (mmol/L)	5.9 (5.2, 6.8)	6.0 (5.2, 6.8)	5.9 (5.1, 6.8)	0.0026
History of myocardial infarction, no. (%)	578 (4.8)	231 (4.7)	347 (4.8)	1.00
History of stroke, no. (%)	262 (2.2)	119 (2.4)	143 (2.0)	0.082
Ferritin (μg/L)	80.0 (42.0, 142.4)	163.6 (125.8, 237.0)	49.0 (28.0, 72.0)	<0.001
TSAT (%)	28 (21, 35)	30 (24, 38)	26 (20, 33)	<0.001
hsCRP (mg/L)	1.3 (0.7, 2.8)	1.4 (0.8, 3.0)	1.2 (0.6, 2.7)	<0.001

BMI, body mass index; hsCRP, high-sensitive C-reactive protein; TSAT, transferrin saturation.
Baseline characteristics of the overall cohort as well as stratified by absolute iron deficiency. The calculation of proportions does not include missing values in the denominator. The P-value was calculated to compare individuals with absolute iron deficiency with individuals without absolute iron deficiency.

Table 2 Baseline characteristics stratified by severe absolute iron deficiency

	No severe absolute iron deficiency (N = 10 175)	Severe absolute iron deficiency (N = 1989)	P-value
Men, no. (%)	5095 (50.1)	398 (20.0)	<0.001
Age (years)	60 (46, 68)	46 (39, 64)	<0.001
Daily smoker, no. (%)	2480 (24.5)	476 (24.0)	0.68
Prevalent diabetes, no. (%)	491 (4.9)	46 (2.3)	<0.001
Hypertension, no. (%)	4847 (47.9)	661 (33.4)	<0.001
BMI (kg/m^2)	26.4 (24.0, 29.3)	24.7 (22.4, 27.7)	<0.001
Systolic blood pressure (mmHg)	132 (119, 147)	123 (112, 142)	<0.001
Total cholesterol (mmol/L)	6.0 (5.2, 6.9)	5.7 (4.9, 6.5)	<0.001
History of myocardial infarction, no. (%)	501 (4.9)	77 (3.9)	0.050
History of stroke, no. (%)	236 (2.3)	26 (1.3)	0.0058
Ferritin (μg/L)	95.9 (59.0, 159.0)	18.0 (12.0, 24.0)	<0.001
TSAT (%)	29 (23, 36)	22 (14, 30)	<0.001
hsCRP (mg/L)	1.4 (0.7, 2.9)	1.0 (0.5, 2.3)	<0.001

BMI, body mass index; hsCRP, high-sensitive C-reactive protein; TSAT, transferrin saturation.
Baseline characteristics of the overall cohort as well as stratified by severe absolute iron deficiency. The calculation of proportions does not include missing values in the denominator. The P-value was calculated to compare individuals with severe absolute iron deficiency with individuals without severe absolute iron deficiency.

Table 3 Baseline characteristics stratified by functional iron deficiency

	No functional iron deficiency (N = 4339)	Functional iron deficiency (N = 7825)	P-value
Men, no. (%)	2899 (66.8)	2594 (33.2)	<0.001
Age (years)	60 (48, 68)	59 (44, 68)	<0.001
Daily smoker, no. (%)	926 (21.4)	2030 (26.1)	<0.001
Prevalent diabetes, no. (%)	256 (6.0)	281 (3.6)	<0.001
Hypertension, no. (%)	2166 (50.1)	3342 (43.0)	<0.001
BMI (kg/m^2)	27.1 (24.8, 30.0)	25.5 (23.1, 28.5)	<0.001
Systolic blood pressure (mmHg)	132 (120, 146)	130 (116, 147)	<0.001
Total cholesterol (mmol/L)	6.0 (5.2, 6.8)	5.9 (5.1, 6.8)	0.069
History of myocardial infarction, no. (%)	203 (4.7)	375 (4.8)	0.81
History of stroke, no. (%)	101 (2.3)	161 (2.1)	0.36
Ferritin (μg/L)	167.0 (127.0, 245.8)	52.0 (29.0, 77.0)	<0.001
TSAT (%)	32 (26, 39)	25 (18, 32)	<0.001
hsCRP (mg/L)	1.4 (0.7, 2.8)	1.3 (0.6, 2.9)	0.028

BMI, body mass index; hsCRP, high-sensitive C-reactive protein; TSAT, transferrin saturation.
Baseline characteristics of the overall cohort as well as stratified by functional iron deficiency. The calculation of proportions does not include missing values in the denominator. The P-value was calculated to compare individuals with functional iron deficiency with individuals without functional iron deficiency.

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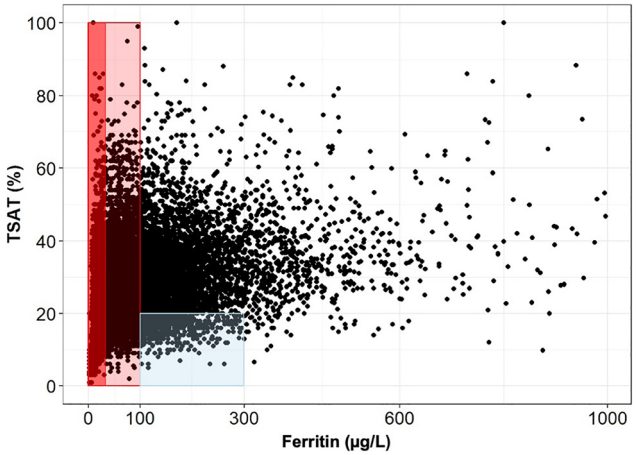
Figure 1 Scatterplot of ferritin and transferrin in the study cohort. Each dot represents one unique individual. The red area marks individuals meeting the criteria of absolute iron deficiency only. The blue area marks individuals meeting the extended criteria of functional iron deficiency only. Individuals with ferritin ≥ 1000 μg/L are not shown (*N* = 32). TSAT, transferrin saturation.



Association of iron deficiency with incident cardiovascular diseases and mortality

During a median follow-up of 13.3 years, 2212 individuals (18.2%) died. Of these, a total of 573 individuals (4.7%) died from a cardiovascular cause. A total of 1033 individuals (8.5%) were diagnosed with incident CHD during the follow-up. Incident stroke was diagnosed in 766 (6.3%) individuals.

After adjustment for multiple confounders, AID was not associated with all-cause mortality [hazard ratio (HR) 1.08, 95% CI 0.98–1.19, $P = 0.12$] or with cardiovascular mortality (HR 1.22, 95% CI 1.00–1.48, $P = 0.05$). AID was associated with incident CHD (HR 1.20, 95% CI 1.04–1.39, $P = 0.01$), but not with incident stroke (HR 1.11, 95% CI 0.94–1.31, $P = 0.22$).

Severe AID was significantly associated with all-cause mortality (HR 1.28, 95% CI 1.12–1.46, $P < 0.01$) but was not associated with cardiovascular mortality (HR 1.01, 95% CI 0.76–1.344, $P = 0.95$) and also not with incident CHD (HR 1.22, 95% CI 1.00–1.50, $P = 0.05$) or incident stroke (HR 1.10, 95% CI 0.87–1.40, $P = 0.42$).

Functional iron deficiency was significantly associated with all-cause mortality (HR 1.12, 95% CI 1.01–1.24, $P = 0.03$), with cardiovascular mortality (HR 1.26, 95% CI 1.03–1.54, $P = 0.03$), with incident CHD (HR 1.24, 95% CI 1.07–1.43, $P < 0.01$), but not with incident stroke (HR 1.15, 95% CI 0.97–1.36, $P = 0.12$). Plots displaying the results for each adjustment and outcome are shown in *Figure 2*.

Population attributable fraction

Within 10 years of follow-up, 5.4% of all-cause deaths were attributable to FID (e.g. 5.4% of these deaths would presumably not have occurred if all individuals had the risk of those without FID at baseline) and 11.7% of cardiovascular deaths. For AID, a lower PAF for all-cause death and cardiovascular death was observed (3.5% vs. 5.4%; 9.7% vs. 11.7%), with the respective CIs containing zero; regarding severe AID, the PAF for all-cause death was lower than the PAF of FID (2.4% vs. 5.4%), and there was only a very small PAF of severe AID for cardiovascular death (with confidence limits containing zero).

The estimated PAF for incident CHD of FID was higher than for AID (10.7% vs. 8.8%), but neither the PAF of severe AID for incident CHD nor the PAFs for AID, severe AID, or FID for stroke could be distinguished from zero (*Table 4*).

Discussion

In this pooled analysis of European population-based cohorts, prevalence of FID was high. FID was associated with CHD, all-cause mortality, and cardiovascular mortality; and 10.7% of the CHD incidence, 5.4% of all deaths, and 11.9% of all cardiovascular deaths within 10 years of follow-up were attributable to FID. Interestingly, AID was only associated with CHD and severe AID only with all-cause mortality, with an overall low PAF for the respective endpoints. These findings indicate that FID, but not AID or severe AID, is an important risk factor for CVD and mortality at the general population level.

Diagnosis of iron deficiency in the context of cardiovascular diseases

Historically, ID has been mainly referred to as a state of severe iron depletion with subsequent anaemia or skeletal muscle dysfunction.[2] The diagnosis of iron depletion (AID or severe AID) has most often been based on very low ferritin values. However, ferritin reflects only iron stored inside cells and does not allow for an assessment of the utilized iron. In contrast, TSAT reflects the utilized iron pool and should hence be measured to allow for a comprehensive evaluation of iron status.[3] This is particularly useful in individuals with clinically apparent or even subclinical CVD. These are known to coincide with a systemic inflammatory response.[17,18] This inflammatory response can obscure ferritin levels as ferritin is also an acute-phase reactant and hence is elevated in inflammation. Consequently, measuring only ferritin to assess ID might be misleading in individuals with clinically apparent or subclinical CVD.

Association of iron deficiency with outcome in the general population

The present study sought to evaluate the association of AID, severe AID, and FID with incident CVD and mortality in the

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Figure 2 Hazard ratios of absolute, severe absolute, and functional iron deficiency for all-cause mortality, cardiovascular mortality, coronary heart disease, and incident stroke. Multivariable Cox regression adjusted for (I) age, sex, and centre; (II) plus smoking, cholesterol, and systolic blood pressure; (III) plus diabetes and body mass index; (IV) plus high-sensitive C-reactive protein. CI, confidence interval; HR, hazard ratio.

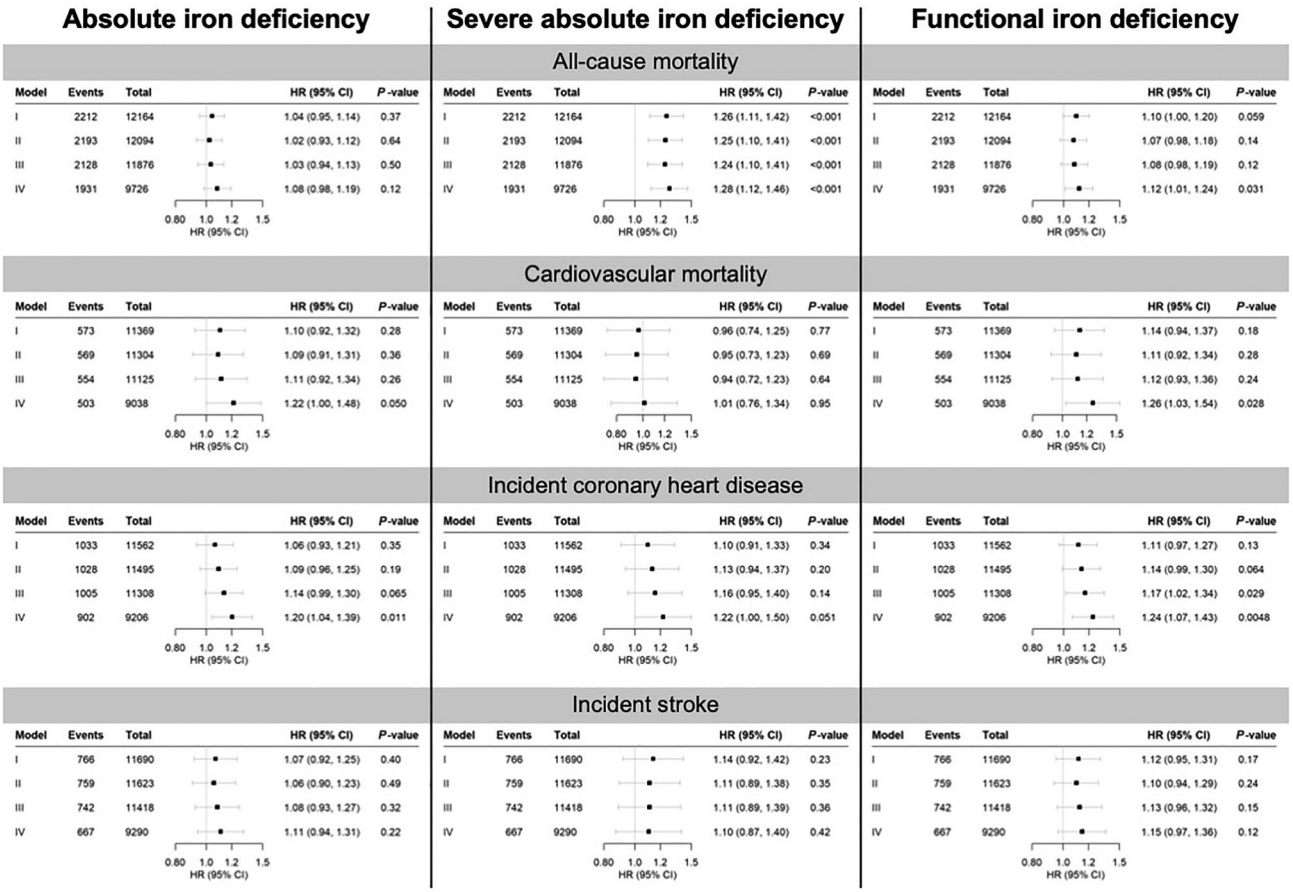

Table 4 Population attributable fraction of iron deficiency

Endpoint	AID PAF (95% CI)	Severe AID PAF (95% CI)	FID PAF (95% CI)
All-cause death	3.5 (−1.0; 8.1)	2.4 (1.2; 3.6)	5.4 (0.3; 10.3)
Cardiovascular death	9.6 (−0.1; 19.3)	−0.6 (−3.6; 2.3)	11.7 (1.9; 21.7)
Coronary heart disease	8.8 (1.6; 15.8)	1.6 (−0.6; 3.7)	10.7 (2.7; 18.7)
Stroke	5.2 (−2.8; 13.5)	0.6 (−1.8; 3.2)	7.4 (−1.4; 16.5)

CI, confidence interval.
Ten-year population attributable fractions (PAFs) for absolute, severe absolute, and functional iron deficiency were calculated (AID, severe AID, and FID, respectively). Separate models were computed for absolute iron deficiency, severe absolute iron deficiency, and functional iron deficiency. Adjustments were made for age, sex, smoking, total cholesterol, systolic blood pressure, diabetes, body mass index, log (high-sensitive C-reactive protein), and study centre. Death from other causes was used as a competing risk for all outcomes other than all-cause mortality.

general population. First of all, ID was highly prevalent in the study cohort, with an observed prevalence of 60.0% for AID, 16.4% for severe AID, and 64.3% for FID. The observed prevalence is well in the range of the prevalence of ID in patients with chronic disease such as heart failure, chronic kidney disease, or inflammatory bowel disease.[19,20]

Secondly, FID was not only significantly associated with CHD but also with all-cause and cardiovascular mortality after adjustment for multiple relevant confounders. AID had a similar association with cardiovascular mortality, although not statistically significant, and no evidence of association with all-cause mortality and was also associated with incident CHD. On the other hand, severe AID was only associated with all-cause mortality, but not with CHD or cardiovascular mortality. These findings are in line with two previous studies, which could show an association of TSAT alone with all-cause and

cardiovascular mortality in the general population.[21,22] Similarly, FID was also associated with all-cause mortality in a recent study based on a German population-based cohort from our group, although this study also indicated an association between AID and all-cause mortality.[23] Overall, these findings highlight the importance of TSAT/FID as a cardiovascular risk marker at the general population level.

Interestingly, presented HRs of FID were modest in the baseline model although the association was greater and had increased statistical significance with increasing levels of adjustment. Importantly, individuals with ID appear to be healthier than individuals without ID at baseline. Hence, adjustment corrects this distribution, revealing a stronger association of FID with mortality. In this regard, it can be speculated that the higher percentage of younger females impacted the results. Pre-menopausal women might have ID due to blood loss during menstruation.[24] They constitute a subpopulation that is likely to be healthier than men of same age. Thus, inclusion of pre-menopausal women in the present analysis might explain the shift of the HRs. However, exclusion would likely result in a stronger association of ID with outcomes.

The large database used for this analysis, the consistent endpoint harmonization in the MORGAM data centre, and the adjustment for multiple relevant confounders support the reliability of the presented findings. Age, sex, smoking, cholesterol, and SBP were chosen for the adjustment based on the widely used *European Society of Cardiology* risk score.[25] Diabetes and BMI were chosen because of their strong association with CVD and mortality.[26–28] Furthermore, hsCRP has been added in the adjustment to account for the close relationship of ID with inflammatory diseases.[20]

Ultimately, these findings indicate that FID seems to be more reliable as a cardiovascular risk factor and might be more specific to identify at-risk individuals than AID or severe AID. A potential reason for this might be that individuals with apparent or subclinical CVD have a certain degree of inflammation that elevates ferritin levels and therefore mitigates against ID diagnosis based on ferritin-only definitions. This would lead to misclassification and might explain the lack of a coherent association between AID/severe AID and cardiovascular endpoints. On the other hand, assessing FID, and thereby assessing utilized iron as well as stored iron, seems to provide a more reliable diagnosis of ID.

Implications

The definition of FID based on ferritin and TSAT has recently been used by two randomized, placebo-controlled trials of iron supplementation in patients with heart failure. These trials showed that iron supplementation improves functional endpoints in individuals with FID irrespective of baseline haemoglobin levels.[8,29] Based on these findings, the 2016 European Society of Cardiology guidelines have implemented a recommendation for iron supplementation in heart failure patients.[30] The present analysis transfers the association of FID with outcome in diseased cohorts to the apparently healthy general population. Based on the estimated attributable risks, about 5% of all 10 year deaths, about 12% of cardiovascular deaths, and about 11% of incident CHD would not have occurred if FID had been absent at baseline.

Therefore, iron supplementation might be an option to address the early prevention of CVD in the general population. This has potential for clinical benefit, as oral iron supplementation is already recognized as a low-cost intervention in older individuals.[31] Given the high prevalence of FID in this study, food fortification programmes might also be an option to provide a nation-wide amelioration of ID. Additionally, the newer forms if intravenous iron could be targeted towards individuals at high risk/with a high level of ID, as these are linked to far less adverse events and therefore provide a favourable efficacy/safety profile.

Ultimately, these results underline the importance of TSAT measurement for diagnosis of ID and highlight FID as a relevant risk factor for CVD and mortality in the general population. Furthermore, these findings call for further research into this topic, especially as several interventions are available to address FID/CVD at a general population level.

Limitations

The presented data were based on three European population-based cohorts and thus might not be generalizable to other populations. Secondly, information on iron supplementation during the study period was not available, which might have impacted the results. Thirdly, haemoglobin levels at baseline were not available, and results were not adjusted for the potential co-occurrence of anaemia. Fourth, although the regression models were adjusted for relevant confounders, this does not rule out confounding due to parameters not examined in the study, such as poverty. Thus, the morbidity and mortality attributable to AID, severe AID, and FID could be exaggerated. Fifth, iron status was only assessed once at baseline, so we do not know the impact on dynamic risk if AID, severe AID, and FID varied over the life course. Sixth, ferritin, TSAT, and hsCRP were measured by different methods in the three cohorts, which might have impacted the comparability of our findings. While this is an observational study, our findings, nevertheless, align with recent Mendelian randomization analyses that link iron status with CVD risk.[32]

Conclusions

Functional iron deficiency was highly prevalent in this pooled analysis of European population-based cohorts. FID was

independently associated with CHD, all-cause mortality, and cardiovascular mortality after adjustment for relevant confounders, whereas AID was only associated with CHD and severe AID only with all-cause mortality. Attributable risks of FID for the tested outcomes were higher than those of AID or severe AID (e.g. more events could have been prevented if individuals would have been free of FID at baseline).

Conflict of interest

B.S.: None related to the current work. Outside: Funding by the German Research Foundation and by the Else Kröner-Fresenius-Stiftung and speakers fee by AstraZeneca and Abiomed.

V.S.: None related to the current work. Outside: Served in advisory boards for Novo Nordisk and Sanofi and has ongoing research collaboration with Bayer Ltd (all unrelated to the present study).

S.S.: None related to the current work. Outside: Speakers honoraria from Actelion Pharmaceuticals Ltd.

W.K.: None related to the current work. Outside: Personal fees for consulting from AstraZeneca, Novartis, DalCor, Kowa, and Amgen and grants and non-financial support from Roche Diagnostics, Beckmann, Singulex, and Abbott.

S.B.: None related to the current work. Outside: Research funding from Abbott Diagnostics, Bayer, SIEMENS, Singulex, and Thermo Fisher; speakers fee from Abbott, Abbott Diagnostics, Astra Zeneca, Bayer, AMGEN, Medtronic, Pfizer, Roche, SIEMENS Diagnostics, SIEMENS, and Thermo Fisher; and honoraria as a member of Advisory Boards and for consulting for Bayer, Novartis, and Thermo Fisher.

M.K.: Research funding, honoraria for lectures, and personal fees for consulting from Vifor Pharma. Outside the submitted work: Personal fees and honoraria for consulting and lectures from AstraZeneca, Adrenomed, Amgen, and Sanofi and research funding by the European Research Area Network (ERA-Net) (PREMED-CAD) (grant no. FKZ01KL1807).

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The other authors do not report a conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Missing values at baseline.

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