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Original Research Article

# Association between hemoglobin levels and mild cognitive impairment in generally healthy European community-dwelling older adults: a 3-year prospective analysis of the DO-HEALTH trial

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A B S T R A C T

**Background:** Although numerous cross-sectional studies have examined the relationship between anemia and cognitive impairment or dementia in older adults, data from larger longitudinal studies, especially in generally healthy older adults, are limited.

**Objectives:** To investigate the associations between baseline hemoglobin levels, anemia, and mild cognitive impairment (MCI) over 3 y in generally healthy older adults.

**Methods:** This is an observational analysis of the 3-y DO-HEALTH trial, a double-blind, randomized controlled trial including 2157 European community-dwelling adults age 70+. Cognition was assessed at baseline, 12, 24, and 36 mo using the Montreal Cognitive Assessment (MoCA). MCI was defined as a MoCA score <26 at 2 consecutive time points. The exposures were the quintiles of hemoglobin levels and anemia at baseline. Logistic regression models based on generalized estimating equations controlled for age, sex, prior falls, study site, treatment allocation, body mass index, number of comorbidities, smoking status, use of iron supplements, alcohol consumption, renal function, and vitamin B12 levels.

**Results:** A total of 2150 participants were included in the analyses (mean age of 74.9 y; 61.7% females). Compared with the lowest quintile, participants in all higher quintiles had a significantly lower odds of MCI: 2nd = 34% lower odds of MCI [odds ratio (OR) = 0.66; 95% confidence interval (CI): 0.47, 0.93; $P = 0.02$], 3rd = 39% (OR = 0.61; 95% CI: 0.43, 0.86; $P = 0.005$), 4th = 44% (OR = 0.56; 95% CI: 0.39, 0.82; $P = 0.003$), and 5th = 36% (OR = 0.64; 95% CI: 0.43, 0.97; $P = 0.03$). For anemia, there was no association with the odds of MCI over time.

**Conclusions:** Baseline hemoglobin levels >130 g/L were associated with reduced odds of MCI over 3 y. Although this study does not establish causality, it suggests further investigations in monitoring and managing hemoglobin levels, even in generally healthy older adults.

This trial was registered at clinicaltrials.gov as NCT01745263.

*Keywords*: mild cognitive impairment, hemoglobin, anemia, DO-HEALTH, older adults

## Introduction

Due to increased life expectancy, the proportion of older adults is projected to grow substantially in the coming years [1]. Age is a primary risk factor for declines in core cognitive functions, including memory, attention, visuospatial abilities, and executive functions [2]. Consequently, this demographic shift is expected to substantially increase the number of individuals at risk of cognitive decline and dementia, with the global prevalence of dementia projected to rise from 57 million in 2019 to 153 million by 2050 [3].

In light of the absence of curative treatments for dementia, recent focus has shifted toward the preclinical stages of the disease, where interventions may slow the progression of cognitive decline [4]. Mild cognitive impairment (MCI), the transitional stage between normal

*Abbreviations:* CI, confidence intervals; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; OR, odds ratio; RCI, Reliable Change Index.

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cognitive aging and dementia [5], affects 16%–20% of individuals aged 65 y and older [6]. MCI has been shown to be significantly associated with an increased risk of developing dementia, particularly Alzheimer's disease [7,8], with annual conversion rates to dementia ranging from 10% to 15% [6,9,10]. Within 3 y, the progression rate to Alzheimer's disease has been estimated at 59% for individuals with MCI, compared with 4% among those with normal cognitive function [11].

Identifying modifiable risk factors for cognitive decline is therefore crucial for dementia prevention [12]. A low hemoglobin blood level, given the essential role of hemoglobin in cerebral oxygenation, has been linked to brain health [13–15]. This may be clinically relevant as anemia, commonly defined as a condition characterized by a lower-than-normal hemoglobin concentration, varies in prevalence from 9.2% to 23.9% among community-dwelling older adults, depending on the specific definition used [16–21]. Furthermore, anemia has been associated with other adverse outcomes of accelerated aging including cardiovascular diseases, falls, fractures, hospitalizations [22–24], and increased mortality [25].

Although numerous cross-sectional studies have examined the relationship between anemia and cognitive impairment or dementia in older adults [26,27], data from larger longitudinal studies, especially in generally healthy older adults, are limited [26,27]. Finally, a meta-analysis of observational studies highlighted substantial heterogeneity across studies [27]. Most importantly, very few studies have used hemoglobin levels as an exposure variable, which may allow for a more detailed investigation of risk gradients and potential nonlinear associations [28].

Therefore, the present study aims to investigate the association between quintiles of baseline hemoglobin levels, anemia, and the odds of MCI over a 3-y follow-up in over 2000 generally healthy adults aged 70 y and older enrolled in the DO-HEALTH trial.

## Methods

### Participants and study design

This is a post-hoc observational analysis of the DO-HEALTH clinical trial. DO-HEALTH was a multicenter, double-blind, randomized controlled clinical trial designed to support healthy aging in European older adults [29] (NCT01745263), and was conducted between December 2012 and November 2017. A total of 2157 community-dwelling females and males aged 70 y and older were recruited from 7 centers in 5 European countries: Innsbruck (Austria), Toulouse (France), Berlin (Germany), Coimbra (Portugal) and Zurich, Basel, Geneva (Switzerland). The inclusion criteria were absence of major health events in the 5 y prior to enrollment, sufficient mobility to come to the study centers, and good cognitive function with a Mini-Mental State Examination (MMSE) score of $\geq$24. Additional details about the trial design and main findings were published elsewhere [29,30]. The DO-HEALTH trial was originally powered for its primary outcomes [29,30], and no a priori power calculation was conducted for the present analysis. The Cantonal Ethical Committee of the Canton of Zurich approved this further use of data and biological material study (BASEC Nr. 2021-00913). All participants included in this study signed the informed consent.

### Outcomes

Cognitive function was assessed with the Montreal Cognitive Assessment (MoCA) at baseline and at each yearly clinical visit. The test evaluates short-term memory recall, visuospatial abilities, attention/concentration, abstract reasoning, executive functions, language, and orientation [31]. The MoCA score ranges from 0 to 30, with a higher score reflecting a better cognitive performance [31]. The presence of MCI was defined as a MoCA score $<$26 at 2 consecutive time points, including baseline and any follow-up time point over 3 y, resulting in up to 3 outcome measures for each participant. This approach was adopted to minimize risk of misclassification due to potential fluctuations in cognitive performance. By requiring the criterion to be met on 2 occasions, we ensure that the identification of MCI more accurately reflects sustained declines in cognitive abilities rather than temporary variations in test results. Because the use of the original cut-off to define MCI (MoCA score $<$26 [31]) in clinical routine has been questioned in recent literature [32], we used an additional cut-off to define MCI, MoCA score $<$ 24, in a sensitivity analysis. Furthermore, as described by Sager et al. [33], an additional sensitivity analysis was conducted using the Reliable Change Index (RCI), applied to both MoCA cutoffs, to account for practice effects in cognitive tests over time [34].

### Exposures

The primary exposure was quintiles of baseline hemoglobin levels. Venous blood was collected after an overnight fast by medically trained staff into tubes containing dipotassium EDTA ($K_2$EDTA). Hemoglobin levels (g/L) were measured at the local certified clinical laboratory of each study center using automated hematology analyzers. Automated hematology analyzers used in certified clinical laboratories have wide analytic ranges that reliably cover the physiologic spectrum of hemoglobin concentrations; in this study, no values were flagged as being outside measurable limits. Quality control was ensured by routine daily calibration and internal controls in accordance with manufacturer instructions and laboratory accreditation standards. Baseline anemia was considered as a secondary exposure and defined as hemoglobin levels $<$130 g/L for males and $<$ 120 g/L for females, following the WHO guidelines [35]. In a sensitivity analysis, we also examined sex-specific quintiles of hemoglobin to account for the physiological differences in hemoglobin distributions between males and females.

### Baseline and time-varying covariates

Covariates were selected based on a theory-driven approach, informed by prior literature and established risk factors for both hemoglobin levels and cognitive outcomes. Participants' characteristics, such as age, sex, smoking status, and BMI, and use of iron supplements, were collected at baseline. Baseline alcohol consumption (g/d) and change in alcohol intake (g/d) from baseline at 3 y were derived from the food frequency questionnaire [36]. Comorbidities were assessed at baseline with the self-administered Comorbidity Questionnaire [37]. Vitamin B12 levels (ng/L) and estimated glomerular filtration rate (eGFR) (mL/min per 1.73 $m^2$) were measured at baseline and during each yearly clinical visit.

### Statistical analysis

Baseline demographic and clinical characteristics of the study population are presented overall, and stratified by quintiles of baseline hemoglobin levels. Normally distributed variables are summarized as mean (SD), skewed variables as median (IQR), and categorical variables as *n* (%). The association between baseline quintiles of hemoglobin levels, anemia, and MCI over 3 y was assessed using generalized estimating equations for repeated binary outcomes including unstructured covariance. The models controlled for randomization stratification factors including study site, sex, age,

previous fall, treatment allocation, and time-varying BMI. The additional covariates included in the models were baseline number of comorbidities, baseline smoking status, baseline use of iron supplements, change in alcohol intake (g/d) from baseline at 3 y, time-varying estimated glomerular filtration and time-varying vitamin B12 levels. All the results were expressed as odds ratios (ORs) with their 95% confidence intervals (CIs). Additionally, we included interaction terms between sex, age group (70–74 or 75+), baseline iron status (soluble transferrin receptor levels >28.1 nmol/L or ≤28.1 nmol/L), and the exposures in the different models to assess potential moderation. Subgroup analyses by these variables were planned if significant interactions were identified. Analyses were based on available cases; no imputation was performed for missing data. Statistical analyses involved using SAS version 9.4 (SAS Institute). Two-sided $P$ values $< 0.05$ were considered statistically significant.

## Results

### Baseline characteristics of the study population

A total of 2150 participants with available hemoglobin levels and MoCA scores at baseline were included in the analysis (Supplementary Figure 1), with baseline characteristics presented in Table 1. The mean age was 74.9 y (SD = 4.4), and 61.7% were females. Participants in the highest hemoglobin quintile (quintile 5, 150–189 g/L) were generally younger, more often male, and reported higher alcohol intake compared with those in the lowest quintile (quintile 1, 89–129 g/L). They also had fewer comorbidities, higher eGFR, and lower prevalence of anemia and iron supplement use. The median (IQR) MoCA scores were 26.0 (24.0, 28.0) at baseline, 27.0 (25.0, 29.0) at year 1, 27.0 (25.0, 29.0) at year 2, and 27.0 (25.0, 29.0) at year 3. The prevalence of MCI over the course of the study, based on the different cut-points applied, is detailed in Supplementary Table 1.

### Association between baseline hemoglobin levels and MCI over 3 y

The odds of MCI significantly decreased across the 5 quintiles of baseline hemoglobin levels, indicating a consistent linear trend ($P = 0.03$). Adjusting for age, sex, prior falls, study site, treatment allocation, BMI, number of comorbidities, use of iron supplements, smoking status, alcohol consumption, renal function, and vitamin B12 levels, participants in the second quintile had 34% lower odds of MCI compared with those in the first quintile (OR = 0.66; 95% CI: 0.47, 0.93; $P = 0.02$). Similarly, participants in the third, fourth, and fifth quintiles had reduced odds of MCI by 39% (OR = 0.61; 95% CI: 0.43, 0.86; $P = 0.005$), 44% (OR = 0.56; 95% CI: 0.38, 0.82; $P = 0.003$), and 36% (OR = 0.64; 95% CI: 0.43, 0.97; $P = 0.03$), respectively, all compared with the first quintile (Figure 1 and Supplementary Table 2).

There was no evidence of significant moderation by sex ($P = 0.92$), age ($P = 0.68$), or baseline iron status ($P = 0.25$) in the relationship between hemoglobin levels and MCI. Therefore, no subgroup analyses were conducted.

### Association between baseline anemia and MCI over 3 y

No significant association was found between baseline anemia and MCI (OR = 1.39; 95% CI: 0.89, 2.18; $P = 0.15$). Subgroup analyses were not conducted, as there was no evidence of significant moderation by sex ($P = 0.56$), age ($P = 0.34$), or baseline iron status ($P = 0.61$) in the relationship between anemia at baseline and MCI over 3 y.

### Sensitivity analyses

When using sex-specific quintiles of hemoglobin, the odds of MCI significantly decreased across quintiles, with a $P$ value for linear trend of 0.04. Consistent with the main analysis, participants in higher quintiles generally had lower odds of MCI compared with those in the

**TABLE 1**
Baseline characteristics of the study population, overall and by quintile of hemoglobin levels at baseline

| | Quintile 1 (89–129 g/L) | Quintile 2 (130–136 g/L) | Quintile 3 (137–143 g/L) | Quintile 4 (144–149 g/L) | Quintile 5 (150–189 g/L) | Overall |
|---|---|---|---|---|---|---|
| | $n = 414$ (19.3%) | $n = 464$ (21.6%) | $n = 446$ (20.7%) | $n = 372$ (17.3%) | $n = 454$ (21.1%) | $N = 2150$ |
| Age (y), mean (SD) | 75.9 (5.1) | 74.8 (4.2) | 74.7 (4.4) | 74.4 (4.0) | 74.9 (4.2) | 74.9 (4.4) |
| Females, *n*(%) | 363 (87.7) | 378 (81.4) | 308 (68.9) | 193 (51.9) | 85 (18.7) | 1326 (61.7) |
| Current smoking, *n*(%) | 12 (2.9) | 21 (4.5) | 35 (7.8) | 23 (6.2) | 34 (7.5) | 124 (5.8) |
| Alcohol intake (g/d), median (IQR) | 1.6 (0.0–7.0) | 3.0 (0.8–11.7) | 5.1 (0.9–12.3) | 5.6 (0.8–13.0) | 8.5 (1.9–14.6) | 5.1 (0.8–11.8) |
| BMI (kg/m$^2$), mean (SD) | 26.4 (4.8) | 25.8 (4.5) | 26.2 (4.1) | 26.3 (4.4) | 26.8 (3.6) | 26.3 (4.3) |
| Prior fall, *n* (%) | 195 (47.1) | 200 (43.1) | 192 (43.0) | 156 (41.9) | 157 (34.6) | 899 (41.8) |
| Number of comorbidities,[1] mean (SD) | 2.2 (1.7) | 1.7 (1.4) | 1.7 (1.3) | 1.6 (1.4) | 1.5 (1.2) | 1.7 (1.4) |
| Estimated glomerular filtration rate (mL/min/1.73 m$^2$), mean (SD) | 61.2 (12.3) | 63.1 (10.4) | 63.0 (9.5) | 64.1 (9.8) | 63.0 (9.2) | 62.9 (10.3) |
| Vitamin B12 levels (ng/L), median (IQR) | 364.0 (284.0–494.0) | 383-0 (296.0–492.0) | 383.0 (302.0–487.0) | 389.5 (317.5–502.0) | 380.0 (284.5–492.5) | 380.0 (295.0–4.94.0) |
| Iron deficiency,[2] *n* (%) | 129 (31.5) | 107 (23.3) | 112 (25.2) | 94 (25.3) | 94 (25.3) | 572 (26.8) |
| Use of iron supplements, *n* (%) | 28 (6.8) | 30 (6.5) | 30 (6.7) | 16 (4.3) | 16 (3.5) | 120 (5.6) |
| Hemoglobin levels (g/L), mean (SD) | 122.3 (6.5) | 133.2 (2.0) | 140.0 (2.1) | 146.4 (1.7) | 156.6 (6.5) | 139.7 (12.4) |
| Anemia,[3] *n* (%) | 142 (34.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 142 (6.6) |

[1] Self-reported number of comorbidities was assessed by the Sangha questionnaire, range 0–13.
[2] Iron deficiency was defined by soluble transferrin receptor levels >28.1 nmol/L.
[3] Anemia was defined as hemoglobin <130 g/L for males and <120 g/L for females.

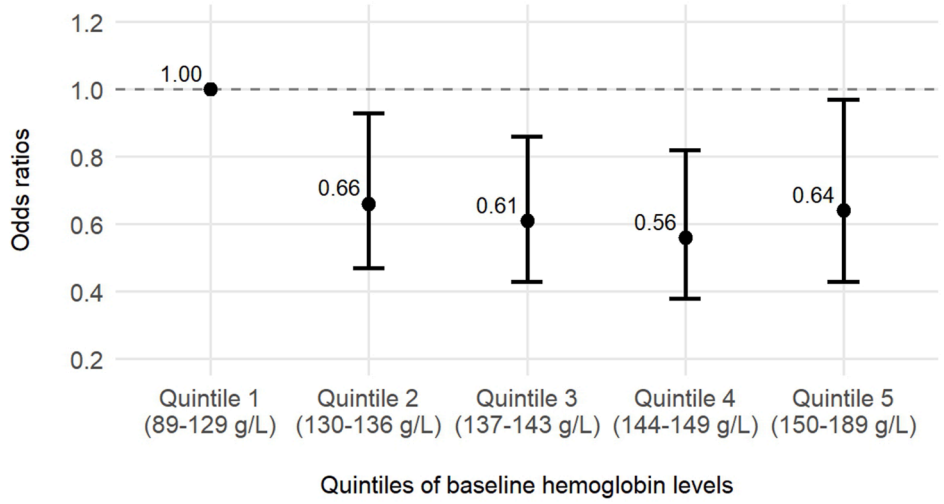


**FIGURE 1.** Association between quintiles of baseline hemoglobin levels and mild cognitive impairment over 3 y. Quintile 1 (reference): $n = 414$; quintile 2: $n = 464$; quintile 3: $n = 446$; quintile 4: $n = 372$; quintile 5: $n = 454$. The model controls for age, sex, prior fall, treatment allocation, study center, and BMI (time-varying covariate), baseline number of comorbidities, baseline use of iron supplements, change in alcohol intake from baseline at 3 y, renal function (time-varying covariate), vitamin B12 levels (time-varying covariate), and baseline smoking status. $P$ value for linear trend: $P = 0.03$. Error bars represent the 95% confidence intervals around the odds ratios (dots) for mild cognitive impairment across quintiles of baseline hemoglobin levels. The dashed line at odds ratio = 1 indicates no association.

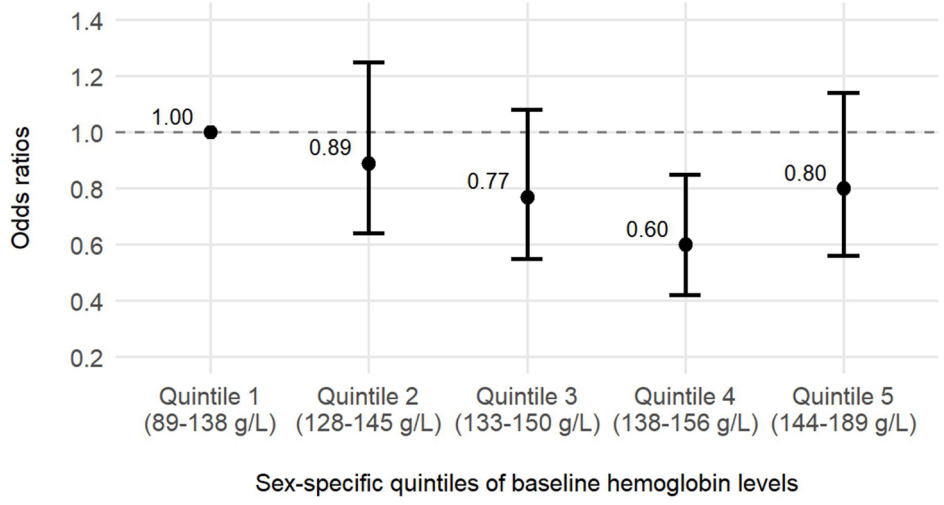


**FIGURE 2.** Association between quintiles of baseline hemoglobin levels and mild cognitive impairment over 3 y—sensitivity analysis using sex-specific quintiles of baseline hemoglobin levels. Quintile 1 (reference): $n = 449$; quintile 2: $n = 393$; quintile 3: $n = 451$; quintile 4: $n = 428$; quintile 5: $n = 434$. The model controls for age, sex, prior fall, treatment allocation, study center, and BMI (time-varying covariate), baseline number of comorbidities, baseline use of iron supplements, change in alcohol intake from baseline at 3 y, renal function (time-varying covariate), vitamin B12 levels (time-varying covariate), and baseline smoking status. $P$ value for linear trend: $P = 0.04$. Error bars represent the 95% confidence intervals around the odds ratios (dots) for mild cognitive impairment across quintiles of baseline hemoglobin levels. The dashed line at odds ratio = 1 indicates no association.

lowest quintile, although individual quintile comparisons did not all reach statistical significance (Figure 2 and Supplementary Table 3). When using a more conservative definition of MCI, the odds of MCI significantly decreased across the 5 quintiles of baseline hemoglobin levels, with a $P$ value for linear trend of $P = 0.009$. Consistent with the main analysis, we found that participants included in the second, third, fourth, and fifth quintiles of hemoglobin levels had significantly lower odds of MCI over 3 y, compared with those included in the first quintile (Figure 3 and Supplementary Table 4). Furthermore, there was no significant association between baseline anemia and the odds of having MCI over 3 y (Supplementary Table 4). When the RCI was used to account for practice effects, the results remained quite consistent with the analyses based on the 2 MoCA cutoffs. Further details are provided in Figures 4 and 5, and Supplementary Tables 5 and 6.

## Discussion

In this study of 2150 generally healthy, community-dwelling adults aged 70 and older, we found that participants in the higher quintiles of baseline hemoglobin levels had lower odds of MCI over 3 y of follow-up, compared with those in the lowest quintile. Notably, participants in the fourth quintile of hemoglobin levels (144–149 g/L) had the lowest

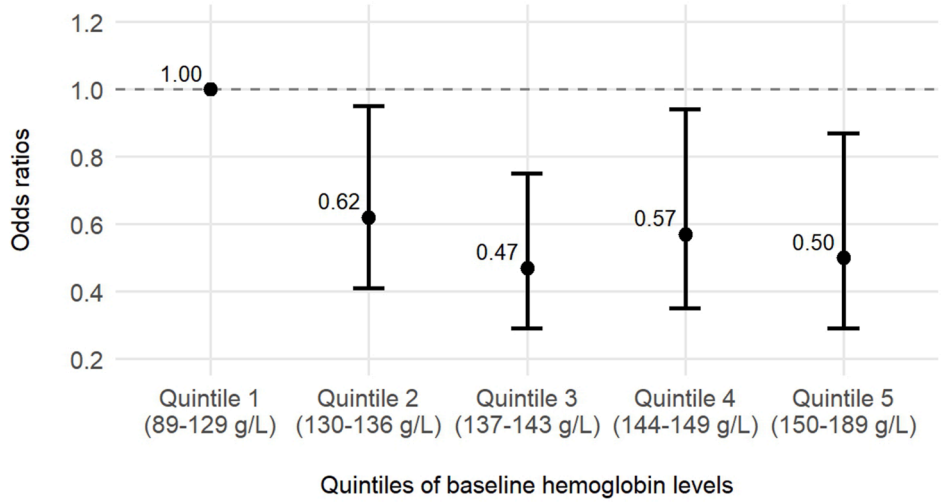


**FIGURE 3.** Association between quintiles of baseline hemoglobin levels and mild cognitive impairment over 3 y—sensitivity analysis using MoCA score <24. Quintile 1 (reference): $n = 414$; quintile 2: $n = 464$; quintile 3: $n = 446$; quintile 4: $n = 372$; quintile 5: $n = 454$. The model controls for age, sex, prior fall, treatment allocation, study center, and BMI (time-varying covariate), baseline number of comorbidities, baseline use of iron supplements, change in alcohol intake from baseline at 3 y, renal function (time-varying covariate), vitamin B12 levels (time-varying covariate), and baseline smoking status. *P* value for linear trend: $P = 0.01$. Error bars represent the 95% confidence intervals around the odds ratios (dots) for mild cognitive impairment across quintiles of baseline hemoglobin levels. The dashed line at odds ratio = 1 indicates no association. MoCA, Montreal Cognitive Assessment.

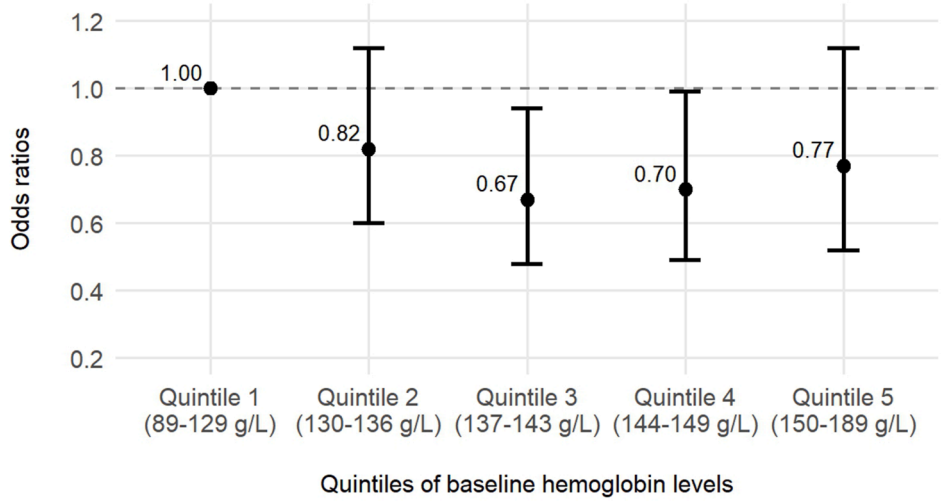


**FIGURE 4.** Association between quintiles of baseline hemoglobin levels and mild cognitive impairment over 3 y—sensitivity analysis using MoCA score < 26 after adjusting for practice effects with the Reliable Change Index. Quintile 1 (reference): $n = 414$; quintile 2: $n = 464$; quintile 3: $n = 446$; quintile 4: $n = 372$; quintile 5: $n = 454$. The model controls for age, sex, prior fall, treatment allocation, study center, and BMI (time-varying covariate), baseline number of comorbidities, baseline use of iron supplements, change in alcohol intake from baseline at 3 y, renal function (time-varying covariate), vitamin B12 levels (time-varying covariate), and baseline smoking status. *P* value for linear trend: $P = 0.11$. Error bars represent the 95% confidence intervals around the odds ratios (dots) for mild cognitive impairment across quintiles of baseline hemoglobin levels. The dashed line at odds ratio = 1 indicates no association. MoCA, Montreal Cognitive Assessment.

odds of MCI in the main analysis. The robustness of these findings was supported by several sensitivity analyses that applied a more stringent cut-point to define MCI, accounted for potential practice effects in the cognitive tests, and used sex-specific hemoglobin quintiles as an alternative exposure definition.

Although the binary classification of anemia did not yield significant findings in our study, examining baseline quintiles of hemoglobin levels revealed a robust association with MCI, aligning more closely with recent studies. In a longitudinal study of 10,918 Chinese community-dwelling adults aged 45 y or older, Qin et al. [38] identified a linear relationship between hemoglobin concentration and global cognitive function, with higher hemoglobin levels linked to better cognitive performance over 2 y of follow-up. Of note, the authors did not find any sex-specific differences in this association. Similarly, Trevisan et al. [28] in a study of 3099 Italian community-dwelling older adults aged 65 y and older, reported a 37% higher risk of cognitive impairment in individuals in the lowest tertile of hemoglobin levels. Notably, this association was significant among

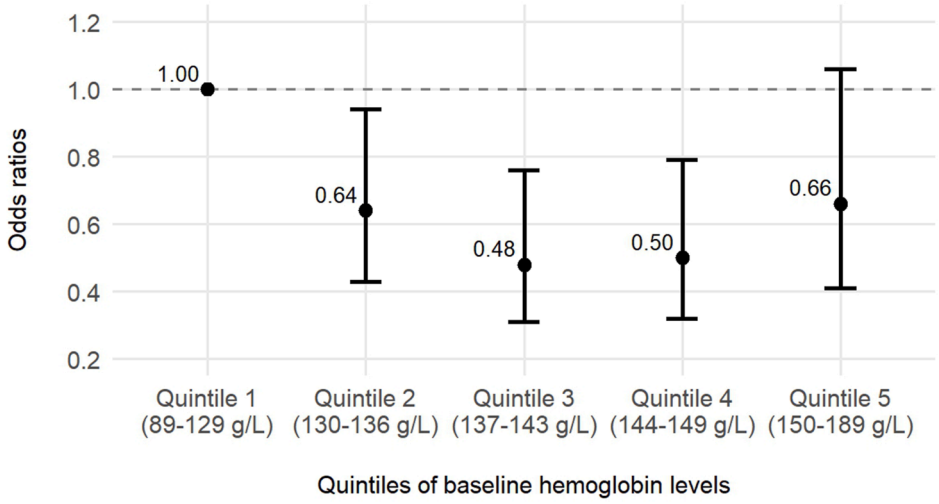


**FIGURE 5.** Association between quintiles of baseline hemoglobin levels and mild cognitive impairment over 3 y—sensitivity analysis using MoCA score < 24 after adjusting for practice effects with the Reliable Change Index. Quintile 1 (reference): $n = 414$; quintile 2: $n = 464$; quintile 3: $n = 446$; quintile 4: $n = 372$; quintile 5: $n = 454$. The model controls for age, sex, prior fall, treatment allocation, study center, and BMI (time-varying covariate), baseline number of comorbidities, baseline use of iron supplements, change in alcohol intake from baseline at 3 y, renal function (time-varying covariate), vitamin B12 levels (time-varying covariate), and baseline smoking status. *P* value for linear trend: $P = 0.04$. Error bars represent the 95% confidence intervals around the odds ratios (dots) for mild cognitive impairment across quintiles of baseline hemoglobin levels. The dashed line at odds ratio = 1 indicates no association. MoCA, Montreal Cognitive Assessment.

males but not females [28]. Interestingly, our study, using quintiles rather than tertiles to better capture the observed nonlinear relationship between baseline hemoglobin levels and the odds of MCI, found no significant moderating role of sex, likely reflecting differences in study populations, confounder adjustments, and cognitive assessment tools (e.g., MMSE or MoCA).

From a pathophysiological point of view, the relationship between hemoglobin levels and MCI seems to have several plausible biological mechanisms. Anemia is often caused by a deficit of erythropoietin due to chronic kidney disease or other chronic conditions, which may impact erythropoiesis, where erythropoietin serves as a critical growth factor [39]. Animal studies demonstrated that erythropoietin mitigates tau protein effects and protects against β-amyloid-induced cell death, with erythropoietin receptors exhibiting neuroprotective properties [40–43]. In clinical studies, it appears that anemia may lead to inadequate cerebral oxygenation [13–15]. Consequently, low levels of hemoglobin may contribute to chronic brain hypoxia increasing risk for cognitive decline [13–15]. Beyond erythropoietin deficits and brain hypoxia, other mechanisms, such as increased oxidative stress [44] and chronic low-grade inflammation [45], commonly associated with anemia, may also play a role in cognitive decline. These pathways warrant further investigation in future studies.

However, the present study did not find any significant association between baseline anemia, defined using the WHO criteria, and MCI. Although the relationship between anemia as a binary exposure and cognitive impairment has been studied in several studies, the findings remain inconsistent [46]. As highlighted by systematic literature reviews and meta-analyses, variations in study designs, sample characteristics (e.g., age, income, education, geography), and adjustments for confounders may partly explain these discrepancies [26,27]. In our study, even after adjusting for a wide range of clinical and lifestyle potential confounders, baseline anemia, defined as hemoglobin < 130 g/L for males and < 120 g/L for females, was not significantly associated with the odds MCI across 3 y. This suggests that anemia as defined by the WHO may not be used as a predictive factor for early cognitive decline in generally healthy older adults, such as those represented by the DO-HEALTH trial. In contrast, Wang et al. [46], recently reported, in an analysis of 207,203 UK Biobank participants aged 60 and over, that anemia as defined by the WHO was associated with faster declines in global cognition over a median follow-up of 9.76 y. Notably, 8209 (3.96%) of those participants had anemia at baseline. The low prevalence of anemia (6.6%) in our study population, which reflects the generally healthy nature of the DO-HEALTH population, may have limited the statistical power to detect an association between anemia and the odds of MCI. In addition, the 3-y follow-up period in DO-HEALTH may have been insufficient to capture the longer-term role of anemia with regard to cognitive decline. Furthermore, although Wang et al. [46] highlighted serum ferritin as a potential mechanism linking anemia and cognition, no data on ferritin were available in that study. In our study, baseline iron status did not significantly moderate the relationship between anemia and MCI, further underscoring the need for more research on the biological pathways linking anemia and cognitive outcomes.

Despite the remaining research gaps, our findings on the relationship between higher hemoglobin levels and reduced odds of MCI may have important implications for public health and clinical practice. Hemoglobin measurement is one of the most commonly performed, simple, and cost-effective laboratory tests in primary care, hospital, and critical care settings [47]. Therefore, integrating hemoglobin assessment and optimization into broader preventive strategies could serve as a valuable approach to identify individuals with low hemoglobin levels who may be at elevated risk of cognitive decline and dementia. Approaches to improve hemoglobin levels in older adults include addressing nutritional deficiencies (e.g., iron, vitamin B12, and folate) [48,49], managing chronic diseases that contribute to anemia [50], and, in selected cases, use of pharmacologic treatments such as erythropoiesis-stimulating agents [51]. Observational studies indicate that erythropoiesis-stimulating agents may enhance cognitive

outcomes in patients with anemia [52,53]. However, intervention studies are necessary to confirm whether managing hemoglobin levels can effectively reduce cognitive impairment and delay decline in older adults.

The strengths of this study include its large, geographically diverse sample and the use of reliable, annually repeated reference-standard cognitive assessments. Adjusting for a wide range of confounders, including vitamin B12 levels and renal function, further strengthened the validity of the observed associations. Additionally, the use of the RCI accounted for potential practice effects in cognitive tests over time. However, several limitations must be noted.

Hemoglobin levels were measured only at baseline; future studies with repeated assessments could clarify whether longitudinal changes in hemoglobin—which are known to occur in older adults [54,55]—are associated prospectively with cognitive outcomes. Although our study included generally healthy participants from 5 European countries, the findings may not be generalizable to populations outside of Europe, to more ethnically diverse populations or to more frailer older adults. Future studies conducted in more ethnically and socioeconomically diverse cohorts could help broaden the applicability of these findings. Additionally, as mentioned earlier, the low prevalence of anemia in our study population may have limited the power to identify a significant association between anemia and MCI. Future studies conducted in populations with a higher anemia burden and with longer follow-up periods could enhance the generalizability of findings and provide greater clarity on the long-term relationship between anemia and MCI. Lastly, due to the observational nature of this study, conclusions regarding causality between hemoglobin levels and cognitive decline cannot be inferred. Despite adjustment for a wide range of sociodemographic and clinical variables, residual confounding cannot be excluded. For example, sleep disturbances, which have been associated with intermittent hypoxia and cognitive outcomes [56,57], were not assessed in DO-HEALTH.

In conclusion, although this study does not establish causal relationships, it highlights the potential importance of maintaining optimal hemoglobin levels for cognitive health in generally healthy adults aged 70 and older. These findings suggest a possible avenue for preventing cognitive decline and dementia in this population, although further research is warranted to clarify causality and underlying mechanisms.

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## Author contributions

The authors' responsibilities were as follows – MW, JF, HAB-F: conceptualized and designed the study; MW: performed the data preparation and analysis, under the supervision of HAB-F; MW, JF, HAB-F: wrote first draft of the manuscript; MM, SG, AE, RWK, MGM: critically reviewed the manuscript; and all authors: read and approved the final manuscript.

## Conflict of interest

HAB-F reports financial support was provided by EMPIRIS Foundation. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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## Data availability

Data described in the manuscript, code book, and analytic code will not be made available to allow primary researchers of the DO-HEALTH Research Group to fully exploit the dataset. The data will be made available to external researchers in a second step according to a controlled access system.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2025.11.005.

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