Hemoglobin levels improve fracture risk prediction in addition to FRAX® clinical risk factors and bone mineral density

[1]Raju Jaiswal, [1,2]Helena Johansson PhD, [1,3]Kristian F. Axelsson MD, PhD, [4]Per Magnusson PhD, [5,6]Nicholas C. Harvey MD, PhD, [1,2]Liesbeth Vandenput PhD, [7,8]Eugene McCloskey MD, PhD, [2,7]John A Kanis MD, [1]Henrik Litsne, [1,9]Lisa Johansson MD, PhD, [1,2,10]Mattias Lorentzon MD, PhD

Affiliations

1. Sahlgrenska Osteoporosis Centre, Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden.
2. Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, VIC, Australia.
3. Region Västra Götaland, Närhälsan Norrmalm, Health Centre, Sweden.
4. Department of Clinical Chemistry, and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden.
5. MRC Lifecourse Epidemiology Centre, University of Southampton, Southampton, UK.
6. NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK
7. Centre for Metabolic Bone Diseases, University of Sheffield Medical School, UK.
8. MRC Versus Arthritis Centre for Integrated research in Musculoskeletal Ageing, Mellanby Centre for Musculoskeletal Research, University of Sheffield, UK.
9. Region Västra Götaland, Department of Orthopedics Sahlgrenska University Hospital, Mölndal, Sweden.
10. Region Västra Götaland, Department of Geriatric Medicine, Sahlgrenska University Hospital, Mölndal, Sweden.

Corresponding author:
Professor Mattias Lorentzon MD, PhD
Head of Geriatric Medicine, Institute of Medicine, Sahlgrenska Academy, Sahlgrenska University Hospital, Mölndal, 431 80 Mölndal, Sweden. Phone: int-46-31-3431979.
E-mail: mattias.lorentzon@medic.gu.se;
ORCID ID: 0000-0003-0749-1431

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Abstract

Context: Anemia and decreasing levels of hemoglobin (Hb) have previously been linked to increased fracture risk, but the added value to FRAX®, the most utilized fracture prediction tool worldwide, is unknown.

Objectives: To investigate the association between anemia, Hb levels, bone microstructure, and risk of incident fracture and to evaluate if Hb levels improve fracture risk prediction in addition to FRAX clinical risk factors (CRFs).

Methods: A total of 2778 community-dwelling women, aged 75-80 years and part of a prospective population-based cohort study in Sweden were included. At baseline, information on anthropometrics, CRFs and falls was gathered, blood samples were collected, and skeletal characteristics were investigated using dual energy x-ray absorptiometry and high-resolution peripheral quantitative computed tomography. At the end of follow-up, incident fractures were retrieved from a regional x-ray archive.

Results: The median follow-up time was 6.4 years. Low Hb was associated with worse total hip and femoral neck bone mineral density (BMD), lower tibia cortical and total volumetric BMD, and anemia was associated with increased risk of major osteoporotic fracture (MOF; hazard ratio 2.04; 95% CI 1.58-2.64). Similar results were obtained for hip fracture and any fracture, also when adjusting for CRFs. The ratio between 10-year fracture probabilities of MOF assessed in models with Hb levels included to not included, ranged from 1.2 to 0.7 at the 10^{th} and 90^{th} percentile of Hb, respectively.

Conclusions: Anemia and decreasing levels of Hb are associated with lower cortical BMD and incident fracture in older women. Considering Hb levels may improve the clinical evaluation of patients with osteoporosis and the assessment of fracture risk.

Introduction

Anemia is a common condition associated with morbidity and mortality, independently and through an array of comorbidities (1). It is characterized by decreased levels of red blood cells and is currently defined as hemoglobin (Hb) levels of <12 g/dl for women and <13 g/dl for men by the World Health Organization (2,3). Interestingly, previous studies have shown an increased risk of fracture with the prevalence of anemia and decreased levels of Hb in both men and women (1,3-10), associations supported by known interactions between processes of bone metabolism and hematopoiesis (11-14). Although the underlying mechanism behind anemia and fracture risk remains to be resolved, anemia has been associated with several risk factors including low bone mineral density (BMD), cardiovascular disease, low general self-rated health status, impaired cognition, low physical function, sarcopenia and falls (1,5,15-19). Additionally, the risk has been particularly apparent in men, with studies on postmenopausal women showing inconsistent results and generally lower risk increases in women as shown in a recent-meta analysis (16). The cause for this sex-specific difference remains unclear.

Over the last few decades, significant improvements in osteoporosis diagnostics, and fracture risk assessment have been accomplished, as well as the development of new and more effective osteoporosis medications (20). However, due to factors such as an ageing demographic and urbanization, the incidence of osteoporotic fracture is projected to increase (21). The fracture risk assessment tool, FRAX, combines age, sex and body mass index (BMI) with a set of clinical risk factors (CRFs) and an optional femoral neck bone mineral density (FN BMD) to estimate the 10-year probabilities

of hip and major osteoporotic fractures (MOF; distal forearm, proximal humerus, clinical spine and hip) (22,23). Since its introduction in 2008, it has been incorporated into more than 80 guidelines for osteoporosis management and is currently the most used fracture prediction tool worldwide (22,24). Although the FRAX-tool considers many CRFs and has numerous advantages, it lacks several potentially important input variables regarding other known risk factors for fracture (22).

Given the previous findings, the primary aim of this study was to investigate further the association between the Hb levels and the risk of incident fracture in older women and to evaluate the contributing effect of Hb levels on 10-year fracture probabilities as calculated by the FRAX-tool. Secondary aims were to analyze the associations between anemia and levels of Hb with variables of BMD and bone microstructure derived from dual-energy x-ray absorptiometry (DXA) and high-resolution peripheral quantitative computed tomography (HR-pQCT).

Method

The study subjects were part of the Sahlgrenska University Hospital Prospective Evaluation of Risk of Bone Fractures (SUPERB) study, and inclusion, cohort characteristics and the used methods have been described in detail in previous publications (25-29).

Subjects

Postmenopausal women aged 75-80 years old at baseline and living in the Gothenburg area, Sweden, were randomly selected from the Swedish national population registry between March 2013 and May 2016. Invitations to participate were sent by letter and telephone to a total of 6832 community dwelling women. The exclusion criteria were not being able to communicate in Swedish, having had bilateral hip replacement, and not being ambulant with or without walking aids. A total of 3028 women were included in the SUPERB study following exclusion of 436 women and 3368 who declined to participate.

Prior to examinations, the participants signed an informed consent form, and the study was approved by the regional Ethics Review Board in Gothenburg, Sweden.

Anthropometrics and questionnaires

Height (to the nearest mm) and weight (to the nearest 0.1kg) were measured using the same standardized equipment for the entire cohort and mean values were used in the analyses. Information regarding the CRFs was obtained through questionnaires and included if the participant had a previous fracture, had a parent with a prior hip fracture, currently smoked tobacco, had been exposed to oral glucocorticoids (of doses corresponding to ≥5 mg of prednisolone for over 3 months in total), and if they had been diagnosed with rheumatoid arthritis. Secondary osteoporosis was defined as having either of diabetes mellitus, hyperthyroidism, chronic liver disease, inflammatory bowel disorder, or premature menopause (<45 years old), and was assessed by asking if the participant had been told by a doctor if they had any of the conditions (yes/no) and at which age menstruations ended. Excessive alcohol intake was defined as 3 or more alcoholic containing drinks per day. Falls were assessed by asking (yes/no) if the participant had experienced a fall during the last 12 months. The FRAX 10-year probabilities for hip and MOF were calculated with and without FN BMD, using the Sweden specific model. Previous osteoporosis treatment was assessed by asking (yes/no) if the participant had ever used bisphosphonates, zoledronic acid, strontium, teriparatide or denosumab and if yes, between which dates.

Blood analyses

Blood samples were collected from all participants at the baseline visit. Plasma and serum samples were immediately stored at - 80 °C until further analysis. Hb was analyzed at the Department of Clinical Chemistry (accredited testing laboratory, Swedac no. 1240), Sahlgrenska University Hospital, Gothenburg, Sweden, using a CN-free Hb method by the ADVIA 2120i system (Siemens Healthcare

Gmbh, Erlangen, Germany) with an analytical range of 0-22.5 g/dl and total coefficients of variation (CVs) of <1.5% at 3 different levels of Hb. Serum albumin and creatinine were analyzed at the Department of Clinical Chemistry (accredited testing laboratory, Swedac no. 1342), Linköping University Hospital, Sweden, and all samples were assayed with reagents from the same batch on a cobas c 701 instrument (Roche Diagnostics Scandinavia AB, Gothenburg, Sweden). Serum albumin was measured by immunoturbidimetry with an analytical range of 3.0-101 g/L and total CVs of ≤4% at 2 different albumin levels. Serum creatinine was measured enzymatically with an analytical range of 5-2700 µmol/L and total CVs of ≤5% at 2 different creatinine levels. Estimated glomerular filtration rate (eGFR) was calculated using the LMR equation (30).

Dual-energy X-ray Absorptiometry (DXA)

The areal bone mineral density (aBMD) was assessed using DXA (Hologic Discovery A, MA, USA). The aBMD (g/cm^2) was analyzed at the non-dominant radius, lumbar spine (L1-L4, excluding fractured vertebrae and/or vertebrae with osteosynthesis material), total hip and femoral neck (FN). The CVs were 3.1% for the radius, 0.68% lumbar spine, 0.83% total hip and 1.3% for the femoral neck.

High-Resolution peripheral Quantitative Computed Tomography (HR-pQCT)

HR-pQCT (XtremeCT, Scanco Medical AG, Switzerland) was used to assess bone microstructure and volumetric bone mineral density (vBMD). The ipsilateral tibia to the non-dominant hand (the contralateral tibia in case of a previous fracture) was analyzed at two sites. A reference line was established at the distal tibia articular plateau. The distal site was at 14% of the tibia length from the reference line, the ultra-distal site was at a standard 22.5mm from the reference line. A total of 110 images were taken over 9.02mm in a proximal direction at each site and were applied to create 3D-models. The parameters calculated were total, cortical, and trabecular cross-sectional areas (mm^2) and vBMD (mg/cm^3), trabecular separation (mm) and thickness (mm), periosteal circumference (mm),

cortical porosity (%) and trabecular bone volume fraction (BV/TV; trabecular bone volume/total bone volume, %). The quality of the images was graded on a scale 1-5, as recommended by the manufacturer, and images with low quality (grade 4-5) at either site were excluded from further analysis. A total of 112 women with images of a low-quality were excluded from the analysis of HR-pQCT variables

Incident fracture evaluation

Evidence of incident fractures in the form of x-ray images and/or x-ray reports were retrieved from medical records or from the regional x-ray archive including Gothenburg and surrounding municipalities. All incident fractures in the regional archive were recorded at the end of follow-up and reviewed by a research nurse and an experienced orthopedic surgeon. The incident fractures were categorized as either hip, MOF or any fracture (including all fracture types, except for fractures of the fingers, toes and skull). No regular x-ray monitoring was conducted, and only incident clinical vertebral fractures identified on examinations with a fracture inquiry were included. Deaths and date of deaths were identified using the regional database Västfolket.

Statistical analyses

Continuous variables were assessed for normality using histograms and tests of skew and kurtosis. Normal distributions were approximated using log transformation for positively skewed variables. The associations between investigated outcome variables and anemia status were analyzed using independent samples *t*-tests, Mann-Whitney U tests, Chi-squared tests, and Fischer's exact tests. The associations to Hb level were investigated using Pearson and Spearman correlation for continuous variables and independent samples *t*-tests for dichotomous variables. The associations between anemia status and Hb level to DXA and HR-pQCT variables were analyzed using independent samples *t*-tests, Pearson correlation and adjusted linear regression models (adjusted for age, weight, and height). The associations between the risk of incident fractures, anemia, and Hb level were assessed using Cox

proportional hazards models with different levels of adjustment: 1) crude, adjusted for age, height and weight, 2) additional adjustments for FRAX CRFs (previous fracture, parent fractured hip, current smoking, oral glucocorticoids, rheumatoid arthritis, secondary osteoporosis, and alcohol intake), FN BMD and falls. Competing risks analysis by Fine and Gray was used to evaluate the risk of incident fracture when considering death as a competing event. A spline Poisson regression model with knots fitted at the 10^{th}, 50^{th} and 90^{th} percentiles was used to study the relationship between fracture risk and Hb levels in more detail (31). The spline functions were second order functions between the breakpoints and linear functions at the tails resulting in a smooth curve. The models were adjusted for age, height and weight with mean cohort values used for each variable (age: 78 years, height 161.9 cm, weight: 69 kg). Additional hazard functions for MOF and death were created using an extension of the Poisson regression model (32,33). The first outcome was counted, and each individual observation period was divided into one-month intervals. The included covariates for MOF were time since baseline, current age, BMI and FRAX CRFs (previous fracture, parent fractured hip, current smoking, oral glucocorticoids, rheumatoid arthritis, secondary osteoporosis (diabetes mellitus, hyperthyroidism, chronic liver disease, inflammatory bowel disorder, or premature menopause), and high alcohol intake). The included covariates for death were time since baseline, current age, BMI, current smoking, and oral glucocorticoids. The Poisson regression hazard functions for MOF and death were also assessed with the additional contribution of Hb levels as spline functions. To calculate the 10-year probability of MOF, the hazard functions were extrapolated in time, from a median follow-up time of 6.4 years to 10 years. Both the hazard function for MOF and death were used, thus adjusting for the increasing risk of death with increasing age. Importantly, the model used to calculate the 10-year probability of MOF was cohort specific (model coefficients derived from the SUPERB-cohort) and therefore only similar but not identical to the model used in FRAX, which used model coefficients from several cohorts (34,35). The 10-year probability of MOF was calculated with and without levels of Hb as a spline function for women aged 75

and 80 years old, with BMI 26 kg/m^2 (cohort mean) and all FRAX CRFs set to no. The level of significance (alpha) applied was p <0.05. The statistical computation was performed using IBM SPSS (version 28, SPSS Inc., Chicago, IL, USA) and STATA (version 17, StataCorp, Texas, USA) for the competing risks analysis.

Results

A total of 2778 (91.7%) women had complete data on Hb, CRFs and FN BMD and were included in the analysis. Of these, the mean Hb was 13.5 g/dl, and 185 women (6.7%) were anemic (Table 1).

Baseline characteristics and associations with anemia status and hemoglobin level

The cohort baseline characteristics are presented in Table 1. Women with anemia had higher relative frequencies of rheumatoid arthritis, secondary osteoporosis, experienced falls in the last 12 months and had higher FRAX probabilities for hip and MOF when assessed with FN BMD. The Hb level was positive ly correlated to age, height, weight and albumin and inversely associated to creatinine and the FRAX 10-year probabilities for hip and MOF, both assessed with and without FN BMD. Hb levels were higher in women who currently smoked but lower in women with a previous fracture, exposed to oral glucocorticoids, had rheumatoid arthritis, and experienced falls (Table 1).

The association of hemoglobin levels to secondary osteoporosis

Hb levels were lower in women with diabetes mellitus and inflammatory bowel disease although the latter was only borderline statistically significant. There was no statistical difference in Hb levels between women with hyperthyroidism, premature menopause or chronic liver disease compared to controls (Table 1).

DXA and HR-pQCT - associations with anemia status and hemoglobin level

The relationship between anemia status and Hb levels to DXA and HR-pQCT variables are presented as crude and adjusted (adjusted for age, weight, and height) associations in Table 2. There were no

significant differences in the mean Hb levels or proportions of women with anemia between the groups of included and excluded women based on image quality grading (data not shown). Anemia was negatively associated with femoral neck and total hip BMD following adjustments for age, weight, and height. No associations between Hb levels and DXA variables remained after adjustment (Table 2). At the distal tibia site following adjustment, women with anemia had higher total area, greater periosteal circumference, and higher cortical porosity, and the Hb level was inversely associated to these variables (Table 2). Also at the distal site, anemia was associated with lower total and cortical vBMD and the Hb level was positively associated to cortical vBMD (Table 2). At the ultra-distal site following adjustment, women with anemia had a higher total area and periosteal circumference and the Hb level was inversely associated to the same variables (Table 2). Also at the ultra-distal site, anemia was associated with a lower cortical area, total and cortical vBMD, and Hb levels were positively associated to total and cortical vBMD and to trabecular thickness (Table 2).

The association of anemia and hemoglobin level to risk of incident fracture and death

The association between anemia, Hb level with the risk of incident fractures are presented in Table 3. The median follow-up time was 6.4 years (interquartile range 5.7-7.3) during which 148 (5.3%) hip fractures, 601 (21.6%) MOFs, 734 (26.4%) any fractures, and 344 (12.4%) deaths occurred. In Cox proportional hazards models adjusted for age, height, weight, FRAX CRFs and FN BMD, the prevalence of anemia was associated with an increased risk for hip fracture (hazard ratio (HR) 1.75 [95% confidence interval (CI) 1.06-2.90]), MOF (HR 1.85 [1.43-2.41]) and any fracture (HR 1.80 [1.41-2.28]; Table 3). In models with identical adjustments but per SD decrease in Hb levels, there was an increased risk for hip fracture (HR 1.19 [1.01-1.39]), MOF (HR 1.22 [1.12-1.32]) and any fracture (HR 1.23 [1.14-1.33]; Table 3). Similar results were obtained when considering death as a competing risk in addition to all adjustments in models by Fine and Gray (Table 3). The hazard ratios did not materially change with the addition of falls (1 or more during the last 12 months) as a covariate to fully adjusted models (HR per SD decrease in

Hb: hip fracture 1.18 [1.00-1.38], MOF 1.21 [1.12-1.31] and any fracture 1.23 [1.14-1.32]). Similarly, the fracture risks were largely unaffected by adding eGFR as an additional adjustment to already fully adjusted models (HR per SD decrease in Hb: hip fracture 1.18 [1.00 to 1.38], MOF 1.22 [1.12 to 1.32] and any fracture 1.24 [1.15 to 1.33]). Likewise, the fracture risks were largely unaffected by adding previous treatment as an additional adjustment to already fully adjusted models (HR per SD decrease in Hb: hip fracture 1.18 [1.01 to 1.39], MOF 1.21 [1.12 to 1.31] and any fracture 1.23 [1.14 to 1.32]). There were minor differences in fracture risks when adding albumin as a covariate to already fully adjusted models (HR per SD decrease in Hb: hip fracture 1.15 [0.97 to 1.35], MOF 1.18 [1.09 to 1.29] and any fracture 1.21 [1.12 to 1.30]). Similar results were obtained when adding diabetes mellitus as a covariate to fully adjusted models (HR per SD decrease in Hb: hip fracture 1.18 [1.01 to 1.39], MOF 1.21 [1.11 to 1.31] and any fracture 1.23 [1.14 to 1.32]).The prevalence of anemia was associated with an increased risk of death (HR 2.06 [1.49-2.86]) and similarly decreasing levels of Hb (HR per SD decrease 1.12 [1.01-1.25]; Table 3). The associations remained largely unaffected following adjustments (adjusted for age, height, weight and FRAX CRFs; Table 3). The adjusted spline regression curves for hip, MOF, and any fracture according to Hb levels revealed no apparent non-linear associations (Figure Legends **Figure 1**). The relationship between the incidence rate of death and Hb levels had its nadir at approximately median Hb 13.6 g/dl from which it increased with increasing levels of Hb (Figure Legends **Figure 1**).

Hb levels and fracture probabilities

For a 75-year-old woman with BMI 26 kg/m^2, no CRFs and without considering FN BMD, the 10-year probability of MOF with Hb included in the model ranged from 25.9% to 14.8% at the 10th and 90th percentile of Hb respectively (Figure 2A and Table 4). This corresponded to a ratio of 1.2 and 0.7, at the 10th and 90th percentile of Hb respectively, when comparing the 10-year probability of MOF assessed in models with Hb included to not included. Similar results were obtained for an 80-year-old woman under the same conditions (Table 4). The relationship between the ratio of probabilities calculated with and without Hb to the level of Hb is illustrated in Figure 2B.

Discussion

In this population-based cohort of older Swedish women, we found an increased risk of fracture with both the prevalence of anemia and decreasing levels of Hb. The increased risk was independent of FRAX CRFs and FN BMD and when considering death as a competing event. Considering Hb levels had a substantial effect on 10-year fracture probabilities where the probability was underestimated in patients with low Hb levels and overestimated in patients with high Hb levels. Anemia and low Hb levels were also associated with BMD at the femoral neck and total hip, as well as with cortical vBMD and porosity of the tibia indicating that bone fragility with low Hb is due to a primarily affected cortical bone.

These results expand the evidence base which currently lacks consensus regarding the association between anemia and fracture in women (1,5-8). A population-based study in Tromsø, Norway including 2775 postmenopausal women reported no increased risk of non-vertebral fracture with anemia or with decreasing Hb level after adjustment for confounders (5). However, in comparison to the present SUPERB cohort, the Norwegian cohort was based on younger post-menopausal women, analyzed the risk for a different category of incident fractures (non-vertebral), and adjusted for different confounders than in the present analysis. As likely result of the lower mean age, only 2.3% of the women were anemic in the Norwegian study, compared to 6.7% in the present study (36). A

possible explanation for the differing results may be an age dependent fracture risk increase due to anemia. Although, another large study of women with a similar age distribution to the Norwegian study found increased risks of hip, spine, and any type of fracture with anemia (1). Most previous studies have analyzed the association of fracture risk with anemia as a dichotomous variable (1,4,8,9), without investigating the Hb levels as a continuous variable. Interestingly, our results of the relationship between Hb levels and fracture risk reveal that there is little to support the use of anemia cut-off levels. Rather, there is a continuous rise in fracture risk with decreasing Hb levels, displaying a close to linear association. This provides support in favor of using the actual Hb level instead of only the anemia diagnosis as a contributing risk factor for fracture. Additionally, anemia cut-off levels are based on statistical cut-offs not linked to any physiological or health outcomes and applying these cut-offs to fracture risk assessment seems to have no basis (37).

Our results indicate that there is a divergence between the risk of fracture and the risk of death as the incidence rate of death seems to increase above the median Hb level. Primary erythrocytosis is well known to be associated with an increased risk of thrombosis and mortality although not very common with a prevalence of approximately 0.4% in women and thus unlikely to entirely explain this discrepancy (38). However, we speculate that the more common type, secondary erythrocytosis, caused by factors such general tissue hypoxia (smoking, obstructive sleep apnea and hypoxic lung disease, etc.) and local renal hypoxia (renal artery stenosis and hydronephrosis, etc.) is likely to, by proxy of its underlying etiologies, explain some of the increased incidence of death (38). Additionally, it seems intuitive that the increased risk of death would act as an increasing competing risk to the fracture risk analysis, explaining lower incidence of fracture. However, this was analyzed in the competing risk analysis by the Fine and Gray method, showing little or no difference in magnitude or significance.

When analyzing skeletal characteristics with traditional DXA methodology we found that women with anemia had lower femoral neck and total hip aBMD but without any associations with Hb levels.

In previously published studies there has been no clear consensus regarding the association with aBMD in cross-sectional analyses, instead stronger associations to aBMD loss have been found (11,12,19,39). There is very limited research on the associations between HR-pQCT variables and anemia with only few studies on subjects with specific conditions such as thalassemia (40). Our study is the first to analyze the associations between bone variables assessed by HR-pQCT and anemia and/or Hb level in a population-based cohort setting. We found associations between anemia, Hb level and predominantly cortical bone variables, such as cortical area, cortical vBMD and cortical porosity. We hypothesize that this association could be due to cortical bone, in comparison to trabecular bone, is more dependent on Hb levels for a sufficient supply of oxygen while the increased vascularization of trabecular bone renders it more independent of Hb levels. This may be a contributing mechanism through which Hb levels affect fracture risk. The lack of associations with traditional DXA derived BMD could be the result of DXA BMD relying on cortical and trabecular BMD, as well as bone size, making it difficult to identify any factor which is predominantly associated with any of these specific traits. In support of our results, a study analyzing variables derived from peripheral quantitative computerized scans (pQCT) of tibia in relation to anemia and Hb levels, found that anemia was negatively associated with total and cortical vBMD, and Hb levels were positively associated to the same variables in addition to trabecular vBMD (18).

The results from the present study have several clinical implications. First, our results indicate that anemia, or more appropriately Hb levels, are significantly and independently associated with fracture risk and thus should be considered as an additional factor when assessing fracture risk in older women. Our results demonstrate that Hb levels contributed to the 10-year probabilities of MOF as calculated by methods similar to those used in the FRAX algorithm. However, it should be emphasized that additional studies confirming our results in patients with a wider range of age and other settings are necessary prior to any general recommendations regarding the use of Hb levels in adjusting 10-year fracture probabilities. If these findings are confirmed, Hb derived multipliers can be used to calculate Hb-adjusted FRAX 10-year probabilities, as previously proposed for adjustment of

FRAX probabilities for oral glucocorticoid use, recent fracture and previous falls (41-43). Analyzing the Hb concentration requires little resources, is part of a standard clinical evaluation and it is likely that many of the individuals being assessed in terms of fracture risk already have a recent Hb result available. Thus, incorporating Hb levels to fracture risk prediction is likely feasible from a resource point of view.

Among the strengths of this study is the size of the cohort, the extensive characterization of participants, with both HRpQCT and DXA, access to data for a large number of CRFs and potential confounders, as well as high-quality fracture outcome data, using x-ray verification of fractures.

The present study also has limitations, including the cross-sectional design relying on single measurements of Hb and BMD, not allowing inferences of causality or reversibility of risk due to Hb levels. Not all participating women in the SUPERB cohort were included in the analyses, due to missing data on Hb levels or due to insufficient image quality of HRpQCT images, which could have affected the results. Unfortunately, none of the collected data made it possible to make any inferences about different etiologies of anemia and fracture risk. Additionally, the study is limited in the fact that no data on hematological disorders was known.

In conclusion, anemia and decreasing levels of Hb are associated with lower BMD, worse cortical bone traits, and incident fracture, independently of FRAX CRFs and BMD in older women. Considering Hb levels may improve the clinical evaluation of patients with osteoporosis and assessment of fracture risk.

Data availability

Data cannot be made publicly available for ethical and legal reasons. Such information is subject to legal restrictions according to national legislation. Specifically, in Sweden confidentiality regarding personal information in studies is regulated in the Public Access to Information and Secrecy Act (SFS 2009:400). The data underlying the results of this study might be made available upon request, after an assessment of confidentiality. There is thus a possibility to apply to get access to certain public documents that an authority holds. In this case, the University of Gothenburg is the specific authority that is responsible for the integrity of the documents with research data. Questions regarding such issues can be directed to the head of the Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. Contact information can be obtained from medicin@gu.se.

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Figure Legends

Figure 1. The relationship between hemoglobin level (Hb) and incidence rates for fracture and death. Spline Poisson regression curves (continuous lines) with 95% CI (dashed lines) adjusted for age, height and weight are shown for hip fracture (**A**), major osteoporotic fracture (MOF) (**B**), any fracture (**C**) and death (**D**).

Figure 2. The contribution of the hemoglobin (Hb) level to 10-year probability of a major osteoporotic fracture (MOF).

The five Hb points denotes min/max, 10^{th}/90^{th} percentile and median values. The probabilities are derived from extended Poisson regression models including age, BMI, FRAX clinical risk factors (previous fracture, parent fractured hip, current smoking, oral glucocorticoids, rheumatoid arthritis, secondary osteoporosis, and alcohol intake) and Hb as a spline function.

A: 10-year probability of a MOF in a 75-year-old woman according to Hb level. The dashed line denotes probabilities calculated without Hb, the continuous line denotes the probabilities derived from the model incorporating Hb. In the models, BMI is set to 26 kg/m^2 and all other clinical risk factors are set to no.

B: The ratio between the 10-year probability of MOF with Hb and without considering Hb, shown for women at age 75 (continuous line) and 80 (dashed line) years. In the model, BMI is set to 26 kg/m^2 and all other clinical risk factors are set to no.

Tables

Table 1. Baseline characteristics and associations to anemia status and hemoglobin levels.

	All (N=2778)	Anemia status			Hb level (g/dl)				
		Anemia (N=185)	No anemia (N=2593)	*p*	Hb *r*	*p*	Cases	Controls	*p*
Age (years)	77.8 ± 1.6	77.6 ± 1.7	77.8 ± 1.6	0.24	**0.04**	**0.03**			
Height (cm)	162.0 ± 5.9	161.2 ± 6.5	162.0 ± 5.9	0.12	**0.06**	**<0.001**			
Weight (kg)	68.8 ± 12.1	68.0 ± 12.6	68.9 ± 12.0	0.35	**0.12**	**<0.001**			
Hemoglobin (g/dl)	13.5 ± 1.1	**11.4 ± 0.6**	**13.7 ± 0.9**	**<0.001**	-	-			
Albumin (g/l)	42.8 ± 2.9	**41.5 ± 3.0**	**42.9 ± 2.8**	**<0.001**	**0.16**	**<0.001**			
Creatinine (µmol/l)[a]	74.9 ± 18.4	**80.6 ± 29.7**	**74.3 ± 18.2**	**<0.001**	**-0.06**	**<0.001**			
Previous fracture	1026 (36.9%)	77 (41.6%)	949 (36.6%)	0.17			**13.5 ± 1.1**	**13.6 ± 1.1**	**0.02**
Family history of fracture	485 (17.5%)	37 (20.0%)	448 (17.3%)	0.35			13.5 ± 1.1	13.5 ± 1.1	0.75
Current smoking	139 (5.0%)	5 (2.7%)	134 (5.2%)	0.14[b]			**13.8 ± 1.2**	**13.5 ± 1.1**	**<0.001**
Oral glucocorticoid exposure	95 (3.4%)	9 (4.9%)	86 (3.3%)	0.26			**13.3 ± 1.2**	**13.6 ± 1.1**	**0.01**
Rheumatoid arthritis	113 (4.1%)	**17 (9.2%)**	**96 (3.7%)**	**<0.001**			**13.1 ± 1.2**	**13.6 ± 1.1**	**<0.001**
Secondary osteoporosis	747 (26.9%)	**69 (37.3%)**	**678 (26.1%)**	**<0.001**			13.5 ± 1.2	13.6 ± 1.1	0.06
Diabetes mellitus	281 (10.1%)	**35 (18.9%)**	**246 (9.5%)**	**<0.001**			**13.4 ± 1.3**	**13.6 ± 1.1**	**0.01**
Hyperthyroidism[c]	144 (5.2%)	11 (5.9%)	133 (5.1%)	0.63			13.5 ± 1.1	13.4 ± 1.1	0.73
Premature menopause (<45 years)[d]	298 (10.7%)	23 (12.5%)	275 (10.7%)	0.45			13.5 ± 1.2	13.5 ± 1.1	0.99
Inflammatory bowel disease	122 (4.4%)	**14 (7.6%)**	**108 (4.2%)**	**0.03**			13.4 ± 1.1	13.6 ± 1.1	0.05
Chronic liver disease	12 (0.4%)	1 (0.5%)	11 (0.4%)	0.56[b]			13.4 ± 1.2	13.5 ± 1.1	0.76
Alcohol (3 or more units/day)	11 (0.4%)	0	11 (0.4%)	1.00[b]			13.9 ± 1.4	13.5 ± 1.1	0.38
Falls, ≥1 the last 12 months	805 (29%)	**71 (38.4%)**	**734 (28.3%)**	**<0.01**			**13.4 ± 1.1**	**13.6 ± 1.1**	**<0.01**
FRAX 10-year probability:									
Hip fracture without BMD, (%)[a]	13.9 ± 11.3	15.2 ± 13.3	13.7 ± 11.2	0.06	**-0.07**	**<0.001**			
Hip fracture with BMD, (%)[a]	7.2 ± 9.2	**8.5 ± 11.5**	**7.1 ± 8.7**	**0.02**	**-0.04**	**0.02**			

MOF without BMD, (%)[a]	37.9 ± 15.4	30.9 ± 15.9	37.6 ± 15.3	0.06	**-0.07**	**<0.001**
MOF with BMD, (%)[a]	19.9 ± 13.4	**22.0 ± 16.2**	**19.7 ± 13.2**	**0.02**	**-0.05**	**<0.05**

Baseline characteristics are presented as means and standard deviations for continuous variables and number of subjects and group percentages in parentheses for categorical variables. Independent samples *t*-tests and Chi-squared tests were used to evaluate differences in means and frequencies across groups of anemia status. Pearson correlation and independent samples *t*-tests were used to analyze the associations to Hb levels. Statistically significant associations are shown in bold. Hb=hemoglobin, BMD=bone mineral density, MOF=major osteoporotic fracture. [a]Median ± interquartile range, Mann-Whitney U test and Spearman correlation, [b]Fischer's exact test, [c]N=2777, [d]N=2752.

Table 2. The association between anemia, hemoglobin level, DXA and HR-pQCT variables.

Dependent variable:	Anemia status					Hb level			
	Anemia (N=185)	No anemia (N=2583)	*p*	Adjusted β (95% CI)	*p*	Hb *r*	*p*	Adjusted standardized β (95% CI)	*p*
DXA									
Lumbar spine aBMD (g/cm^2)	0.96 ± 0.17	0.94 ± 0.18	0.34	0.10 (-0.04 to 0.24)	0.17	0.01	0.80	-0.04 (-0.07 to 0.00)	0.05
Femoral neck aBMD (g/cm^2)	**0.64 ± 0.11**	**0.66 ± 0.11**	**0.02**	**-0.15 (-0.29 to -0.01)**	**0.04**	**0.04**	**0.03**	-0.00 (-0.04 to 0.03)	0.98
Total hip aBMD (g/cm^2)	**0.78 ± 0.13**	**0.80 ± 0.12**	**0.02**	**-0.15 (-0.29 to -0.02)**	**0.03**	**0.06**	**<0.01**	0.02 (-0.02 to 0.05)	0.32
Radius aBMD (g/cm^2)[a]	0.57 ± 0.97	0.58 ± 0.78	0.12	-0.13 (-0.27 to 0.01)	0.07	**0.05**	**<0.01**	0.02 (-0.02 to 0.05)	0.32
HR-pQCT - distal tibia									
Total area (mm^2)	**447.0 ± 58.3**	**437.8 ± 58.1**	**0.04**	**0.22 (0.08 to 0.36)**	**<0.01**	-0.00	0.96	**-0.05 (-0.08 to -0.01)**	**0.01**
Cortical area (mm^2)	144.9 ± 26.9	148.1 ± 23.4	0.12	-0.12 (-0.26 to 0.02)	0.10	**0.04**	**0.03**	0.00 (-0.03 to 0.04)	0.96
Total vBMD (mg/cm^3)	**369.3 ± 83.1**	**386.2 ± 77.2**	**<0.01**	**-0.23 (-0.38 to -0.09)**	**<0.01**	**0.04**	**0.04**	0.03 (-0.01 to 0.07)	0.09
Cortical vBMD (mg/cm^3)	**909.0 ± 43.2**	**915.9 ± 41.6**	**0.03**	**-0.18 (-0.33 to -0.03)**	**0.02**	**0.05**	**0.01**	**0.05 (0.02 to 0.09)**	**0.01**
Trabecular vBMD (mg/cm^3)[b]	92.8 ± 37.3	95.9 ± 34.9	0.26	-0.08 (-0.23 to 0.07)	0.29	0.02	0.37	0.00 (-0.04 to 0.04)	0.99
Periosteal circumference (mm)[c]	**82.5 ± 5.2**	**81.6 ± 5.3**	**0.03**	**0.23 (0.10 to 0.37)**	**<0.001**	-0.00	0.94	**-0.05 (-0.09 to -0.02)**	**<0.01**
Trabecular BV/TV (%)[c]	7.7 ± 3.1	8.0 ± 2.9	0.29	-0.08 (-0.23 to 0.07)	0.30	0.02	0.33	0.00 (-0.04 to 0.04)	0.96
Trabecular thickness (mm)[b]	0.1 ± 0.02	0.1 ± 0.02	0.48	0.05 (-0.10 to 0.20)	0.51	-0.01	0.64	0.02 (-0.02 to 0.05)	0.40
Trabecular separation (mm)[d, e]	0.67 ± 0.40	0.65 ± 0.31	0.11	0.13 (0.01 to 0.27)	0.08	**-0.05**	**0.02**	-0.00 (-0.03 to 0.04)	0.85
Cortical porosity (%)[e, f]	**5.22 ± 3.3**	**4.86 ± 3.2**	**<0.01**	**0.24 (0.09 to 0.39)**	**<0.01**	**-0.05**	**0.01**	**-0.06 (-0.10 to -0.02)**	**<0.01**
HR-pQCT - ultra-distal tibia									
Total area (mm^2)[c]	743.3 ± 110.1	729.3 ± 105.3	0.09	**0.21 (0.09 to 0.33)**	**<0.001**	-0.00	0.97	**-0.06 (-0.09 to -0.03)**	**<0.001**
Cortical area (mm^2)	75.0 ± 24.8	78.5 ± 23.0	0.05	**-0.18 (-0.32 to -0.03)**	**0.02**	**0.04**	**0.03**	0.03 (-0.00 to 0.07)	0.07

Total vBMD (mg/cm^3)[c]	219.8 ± 51.9	226.6 ± 47.6	0.09	**-0.16 (-0.31 to -0.02)**	**0.03**	**0.05**	**0.01**	**0.04 (0.00 to 0.08)**	**0.03**
Cortical vBMD (mg/cm^3)[c]	728.9 ± 77.8	739.8 ± 68.2	0.07	**-0.19 (-0.34 to -0.05)**	**0.01**	0.03	0.11	**0.04 (0.00 to 0.08)**	**0.04**
Trabecular vBMD (mg/cm^3)[c]	144.1 ± 38.4	146.5 ± 35.0	0.37	-0.06 (-0.21 to 0.09)	0.44	**0.05**	**0.01**	0.02 (-0.01 to 0.06)	0.21
Periosteal circumference (mm)[c]	107.0 ± 7.8	105.9 ± 7.7	0.07	**0.22 (0.11 to 0.34)**	**<0.001**	0.00	0.96	**-0.06 (-0.09 to -0.03)**	**<0.001**
Trabecular BV/TV (%)[c]	12.0 ± 3.2	12.2 ± 2.9	0.36	-0.06 (-0.21 to 0.09)	0.42	**0.05**	**0.01**	0.02 (-0.01 to 0.06)	0.21
Trabecular thickness (mm)[c]	0.1 ± 0.0	0.1 ± 0.0	0.36	-0.09 (-0.24 to 0.06)	0.25	0.03	0.11	**0.05 (0.01 to 0.09)**	**0.01**
Trabecular separation (mm)[e]	0.50 ± 0.13	0.49 ± 0.15	0.75	0.00 (-0.14 to 0.15)	0.95	-0.03	0.08	0.01 (-0.02 to 0.05)	0.48
Cortical porosity (%)[b, e]	11.95 ± 5.6	11.88 ± 5.2	0.63	0.05 (-0.11 to 0.20)	0.55	-0.00	0.96	-0.01 (-0.05 to 0.03)	0.67

The association of anemia status and Hb level to DXA and HR-pQCT variables are presented as crude and adjusted values. Crude values are evaluated using independent t-tests and Pearson correlation for anemia status and Hb level respectively. The adjusted beta coefficients are derived from linear regression models adjusted for age, weight, and height. The beta coefficients shown are SD change in dependent variable with the prevalence of anemia and per SD increase in Hb level (standardized beta). Statistically significant associations are shown in bold. Hb=hemoglobin, CI=confidence interval, DXA=dual-energy x-ray absorptiometry, aBMD=areal bone mineral density, HR-pQCT=high-resolution peripheral quantitative computed tomography, vBMD=volumetric bone mineral density, BV/TV=trabecular bone volume/total bone volume.
[a]N=2762, [b]N=2663, [c]N=2665, [d]N=2653, [e]Median ± interquartile range, [f]N=2654.

Table 3. The association of anemia and hemoglobin level to the risk of incident fracture and death.

	Hip (95% CI)	*p*	MOF (95% CI)	*p*	Any (95% CI)	*p*	Death (95% CI)	*p*
Anemia								
Crude	**2.13 (1.30 to 3.48)**	**0.003**	**2.04 (1.58 to 2.64)**	**<0.001**	**2.00 (1.58 to 2.53)**	**<0.001**	**2.06 (1.49 to 2.86)**	**<0.001**
Adjusted	**2.17 (1.33 to 3.56)**	**0.002**	**2.10 (1.63 to 2.72)**	**<0.001**	**2.06 (1.62 to 2.61)**	**<0.001**	**2.10 (1.06 to 1.21)**	**<0.001**
+CRFs	**2.05 (1.24 to 3.37)**	**0.01**	**2.03 (1.56 to 2.63)**	**<0.001**	**1.94 (1.52 to 2.46)**	**<0.001**	**2.07 (1.49 to 2.88)**	**<0.001**
+FN BMD	**1.75 (1.06 to 2.90)**	**0.03**	**1.85 (1.43 to 2.41)**	**<0.001**	**1.80 (1.41 to 2.28)**	**<0.001**		
Adjusted SHR	1.63 (0.97 to 2.76)	0.07	**1.73 (1.32 to 2.28)**	**<0.001**	**1.70 (1.33 to 2.19)**	**<0.001**		
Hb level (per SD decrease)								
Crude	**1.23 (1.04 to 1.44)**	**0.01**	**1.23 (1.13 to 1.33)**	**<0.001**	**1.25 (1.16 to 1.34)**	**<0.001**	**1.12 (1.01 to 1.25)**	**0.04**
Adjusted	**1.23 (1.05 to 1.45)**	**0.01**	**1.24 (1.15 to 1.35)**	**<0.001**	**1.26 (1.17 to 1.36)**	**<0.001**	**1.13 (1.02 to 1.26)**	**0.02**
+CRFs	**1.21 (1.02 to 1.42)**	**0.03**	**1.23 (1.13 to 1.33)**	**<0.001**	**1.24 (1.15 to 1.34)**	**<0.001**	**1.14 (1.02 to 1.27)**	**0.02**
+FN BMD	**1.19 (1.01 to 1.39)**	**0.04**	**1.22 (1.12 to 1.32)**	**<0.001**	**1.23 (1.14 to 1.33)**	**<0.001**		
Adjusted SHR	**1.18 (1.00 to 1.38)**	**<0.05**	**1.20 (1.10 to 1.30)**	**<0.001**	**1.22 (1.16 to 1.31)**	**<0.001**		

The associations of anemia and Hb level to the risk of incident hip fracture, MOF and any fracture are presented as hazard ratios (HR) derived from Cox proportional hazards models. The HR per SD decrease Hb level is shown. Adjusted: adjusted for age, height, and weight. +CRFs: additional adjustments for previous fracture, parental hip fracture, current smoking, oral glucocorticoids, rheumatoid arthritis, secondary osteoporosis, and alcohol intake. +FN BMD: additional adjustment for FN BMD. Adjusted SHR: subdistribution hazard ratios derived from competing risks analysis by Fine and Gray with all the above adjustments. Statistically significant associations are shown in bold. CI=confidence interval, MOF=major osteoporotic fracture, CRFs=clinical risk factors, FN BMD=femoral neck bone mineral density, SHR=subdistribution hazard ratio, Hb=hemoglobin, SD=standard deviation.

Table 4. 10-year probabilities of MOF with and without considering Hb.

Age	10-year probability of MOF	10-year probability of MOF with Hb			Ratio between 10-year probabilities of MOF calculated with and without Hb			Ratio between 10-year probabilities of MOF calculated with Hb at the 10th and 90th percentile to median Hb (13.6)	
		Hb=12.2 (10th perc.)	Hb=13.6 (50th perc.)	Hb=14.9 (90th perc.)	Hb=12.2 (10th perc.)	Hb=13.6 (50th perc.)	Hb=14.9 (90th perc.)	Hb=12.2 (10th perc.)	Hb=14.9 (90th perc.)
75	21.0	25.9	19.8	14.8	1.23	0.94	0.70	1.31	0.75
80	28.2	34.3	27.8	21.6	1.22	0.99	0.77	1.23	0.78

The 10-year probabilities for MOF are derived from extended Poisson regression models extrapolated to 10 years. The probabilities presented are for women aged 75 and 80 years, with cohort mean BMI (26 kg/m^2), no CRFs and without considering FN BMD. The 10-year probabilities of MOF, when Hb is included, are shown for the 10th, 50th and 90th percentiles of Hb. The fourth column presents the MOF probability ratios calculated with Hb included to Hb not included. The fifth column presents the MOF probability ratios with Hb between the 10th and 90th percentile of Hb to median Hb. When Hb is included in a model it contributes as a spline function. All Hb values are g/dl. MOF=major osteoporotic fracture, Hb=hemoglobin.

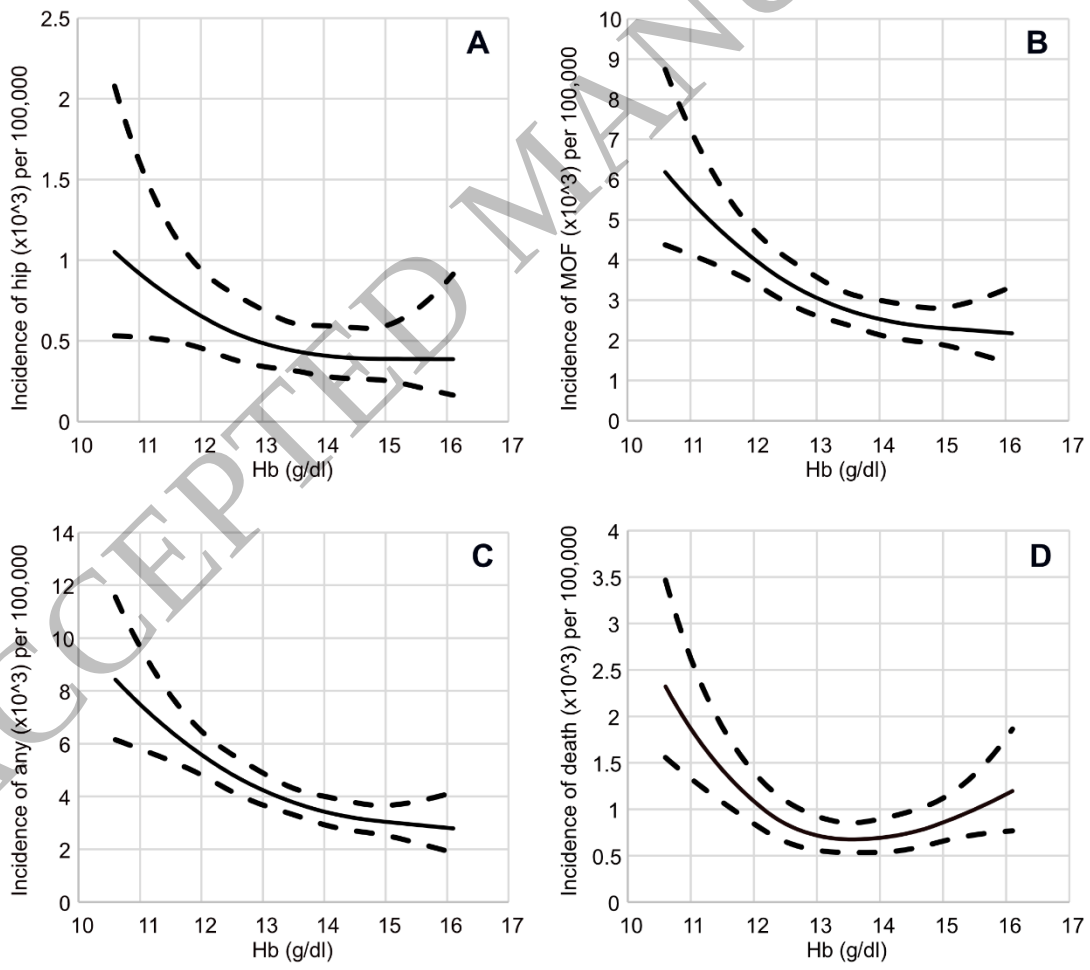

Figure 1
147x130 mm (x DPI)

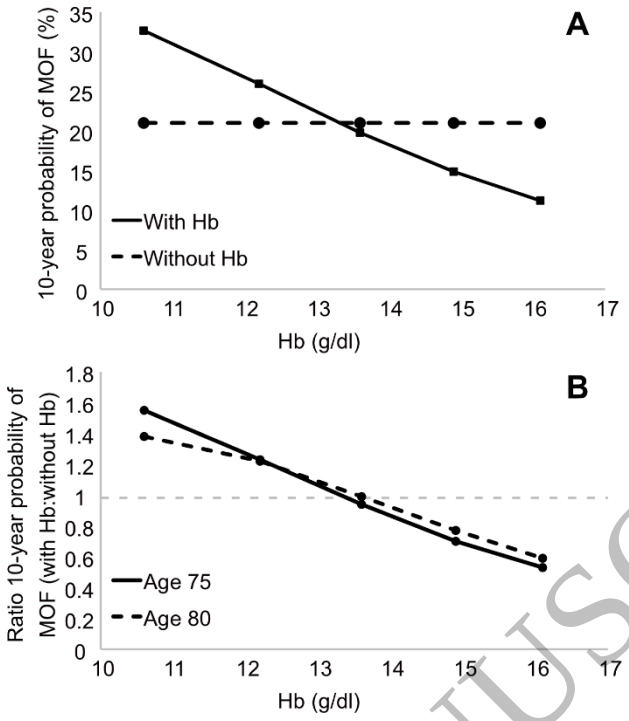

Figure 2
82x95 mm (x DPI)