Predictive value of low ferritin in older persons with anemia with and without inflammation: the Leiden 85-plus Study

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ABSTRACT

Objectives To investigate the association between low ferritin levels and anemia in old age in the presence and absence of inflammation.

Design Prospective population-based observational follow-up study.

Setting Leiden, the Netherlands.

Participants 560 85-year-old individuals participating in the Leiden 85-plus Study. For the present analysis, participants using iron supplements at baseline or during follow-up (n=48) were excluded, resulting in a study population of 512 persons.

Measurements Serum ferritin and C-reactive protein (CRP) concentrations were measured at baseline (age 85 years). Low ferritin was defined as concentrations <20 µg/L for men and <15 µg/L for women. Elevated CRP was defined as concentrations >5 mg/L. Hemoglobin and mean corpuscular volume (MCV) were determined at baseline and annually thereafter for 5 years. Anemia was defined according to World Health Organization criteria.

Results At age 85 years, 122 participants had anemia (23.8%, 122/512). Low ferritin (n=35) was associated with the presence of anemia (OR 2.2 [95% CI 1.1-4.5], adjusted for gender). This association was more pronounced in participants with elevated CRP (OR 7.0 [95% CI 1.4-34.9]) than in participants with normal CRP (OR 1.7 [95% CI 0.7-4.2]). Lowest hemoglobin levels and MCV were found in participants with low ferritin and elevated CRP (one-way ANOVA, p<0.01). In the prospective analyses, low ferritin was associated with an accelerated decline in hemoglobin and MCV, especially in participants with elevated CRP at baseline.

Conclusion In old age, low ferritin is associated with anemia. This association is most prominent in older individuals with signs of inflammation.
INTRODUCTION

Anemia is highly prevalent in the elderly, especially in individuals aged 85 years and over (>20%). Iron deficiency is a common cause of anemia as it is found in more than 15% of older persons with anemia. Serum ferritin levels strongly correlate with body iron stores and are considered the best non-invasive test for the diagnosis of iron deficiency. Ferritin therefore plays a central role in diagnostic and therapeutic algorithms for iron deficiency anemia in clinical practice.

However, ferritin is also an acute phase protein and may be elevated in acute and chronic inflammatory conditions, such as infections, rheumatoid arthritis and cancer. During acute and chronic inflammatory conditions, serum ferritin may not accurately reflect true iron status. Since the prevalence of inflammatory conditions is very high in old age, it is not clear whether low ferritin can be used as a marker of low iron status in old age. Therefore, we investigated the association between low ferritin levels and anemia in old age in the presence and absence of inflammation.

METHODS

Study population and procedures

The present study is embedded in the Leiden 85-plus Study, a population-based prospective follow-up study of 85-year-old inhabitants of Leiden, the Netherlands. Between September 1997 and September 1999, 705 inhabitants of Leiden reached the age of 85 years and were eligible for participation in the study. No other selection criteria regarding health or living arrangements were applied. Fourteen subjects died prior to enrolment in the study and 92 subjects refused to participate. Seven subjects died before blood sample collection and 30 subjects refused blood sampling. For 2 subjects ferritin levels could not be measured due to analytical difficulties. As a result, at baseline, complete laboratory data for the present analyses were available for 560 participants. Participants were visited annually at their place of residence to obtain a blood sample (age 85 through 90 years). Pharmacy records were studied to evaluate the use of iron supplements. Forty-eight participants who used iron supplements at baseline (n=14) or during follow-up (n=34) were excluded from the present study, resulting in a study population of 512 participants. All participants gave their informed consent. The Medical Ethical Committee of Leiden University Medical Center approved the study.

Laboratory measurements

In 2003, ferritin levels were determined in one batch with an immunological assay (E170, Roche, Almere, The Netherlands). Low serum ferritin was defined according to the Dutch guidelines for collaborating medical diagnostic laboratories as ferritin <20 µg/L for men and <15 µg/L for women. CRP levels were measured with a Hitachi 747 automated analyzer (Hitachi, Tokyo, Japan). Elevated CRP was
defined as CRP >5 mg/L. Hemoglobin levels and mean corpuscular volume (MCV) were determined annually (age 85 through 90 years) with an automated analysis system (Coulter Counter, Coulter Electronics, Hialeah, FL). Anemia was defined according to criteria of the World Health Organization (hemoglobin <130 g/L for men and hemoglobin <120 g/L for women). Participants with anemia were further divided into having microcytic anemia (MCV <80 fL), normocytic anemia (MCV 80-100 fL), or macrocytic anemia (MCV >100 fL).

**Comorbidity**

Information on the presence of disease (including stroke, myocardial infarct, severe cognitive impairment, diabetes, Parkinson’s disease, hip fracture, arthritis, obstructive lung disease and malignancy) was obtained from general practitioners, nursing home physicians, pharmacy records and laboratory findings. The presence of severe cognitive impairment was based either on diagnosis by the general practitioner or on a Mini-Mental State Examination score <19. Diabetes mellitus was considered present when diagnosed by the primary care physician, when non-fasting glucose levels were >11.0 mmol/L, or when a participant was taking anti-diabetic medication.

**Data analysis**

Logistic regression models were used to calculate gender-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for the cross-sectional association between ferritin levels and anemia. Men and women were divided separately into four groups based on both ferritin status (normal or low ferritin levels) and inflammatory status (normal or elevated CRP levels) at age 85 years. Differences in mean hemoglobin levels and MCV between these four groups were tested with one-way ANOVA.

Cox proportional hazard models were used to assess the effect of low ferritin levels at age 85 on the development of anemia during follow-up. All models were adjusted for gender. To assess the effect of low ferritin levels at age 85 on changes in hemoglobin levels and MCV during the follow-up period, individual annual changes in hemoglobin levels and MCV were calculated as the difference between the measurement at baseline and the measurement at the last available year of follow-up, divided by the length of follow-up time for each participant (1-5 years). Differences in annual changes in hemoglobin and MCV between participants with low and normal ferritin levels at age 85 were analyzed with linear regression analysis, adjusted for gender.

All data analyses were performed with SPSS 16.0 for Windows (SPSS Inc., Chicago, IL).
Table 1. Characteristics of the study population at age 85 years

<table>
<thead>
<tr>
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<th>n=512</th>
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<tbody>
<tr>
<td>Men</td>
<td>178 (34.8%)</td>
</tr>
<tr>
<td>Institutionalized</td>
<td>87 (17.0%)</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>130 (122-138)</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>92 (88-94)</td>
</tr>
<tr>
<td>Anemia*</td>
<td>122 (23.8%)</td>
</tr>
<tr>
<td>Microcytic anemia (MCV &lt;80 fl)</td>
<td>5 (4.1%)</td>
</tr>
<tr>
<td>Normocytic anemia (MCV 80-100 fl)</td>
<td>114 (93.4%)</td>
</tr>
<tr>
<td>Macrocytic anemia (MCV &gt;100 fl)</td>
<td>3 (2.5%)</td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>75 (38-122)</td>
</tr>
<tr>
<td>Low ferritin†</td>
<td>35 (6.8%)</td>
</tr>
<tr>
<td>Elevated C-reactive protein, mg/L</td>
<td>171 (33.4%)</td>
</tr>
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Number of diseases‡

|                                |                       |
|                                | 127 (25.1%)           |
| One                            | 208 (41.1%)           |
| Two or more                    | 171 (33.8%)           |

Data are presented as median (interquartile range) or n (%).
* WHO criteria: hemoglobin level <130 g/L for men and hemoglobin level <120 g/L for women.
† ferritin <20 µg/L for men and <15 µg/L for women.
‡ including stroke, myocardial infarct, severe cognitive impairment, diabetes, Parkinson’s disease, hip fracture, arthritis, obstructive lung disease and malignancy.

RESULTS

Table 1 describes the characteristics of the study population at baseline (age 85 years, n=512). A total of 178 participants (34.8%) was male and a total of 87 participants (17.0%) was institutionalized in a nursing home or in a home for the elderly. The prevalence of anemia was 23.8% (n=122); 5 participants had microcytic anemia (4.1% of participants with anemia), 114 participants had normocytic anemia (93.4% of participants with anemia), and 3 participants had macrocytic anemia (2.5% of participants with anemia). The median ferritin level was 75 µg/L (interquartile range [IQR] 38-122); 35 participants (6.8%) had low ferritin levels. The prevalence of elevated CRP was 33.4%.
Low ferritin levels were found in 2 participants with microcytic anemia (2/5, 40%), in 12 participants with normocytic anemia (12/114, 11%) and in none of the participants with macrocytic anemia (0/3, 0% [Table 2]). Participants with low ferritin levels had a more than twofold increased risk of having anemia compared to participants with normal ferritin levels (OR 2.2 [95% CI 1.1;4.5]).

<table>
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<tr>
<th>Table 2. Ferritin status in participants with microcytic, normocytic and macrocytic anemia at age 85 years</th>
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</thead>
<tbody>
<tr>
<td>Low ferritin*</td>
</tr>
<tr>
<td>Microcytic anemia†‡</td>
</tr>
<tr>
<td>Normocytic anemia†§</td>
</tr>
<tr>
<td>Macrocytic anemia†</td>
</tr>
</tbody>
</table>

* ferritin <20 µg/L for men and <15 µg/L for women.
† WHO criteria: hemoglobin level <130 g/L for men and hemoglobin level <120 g/L for women.
‡ MCV <80 fl.
§ MCV 80-100 fl.
|| MCV >100 fl.

Since the association between low ferritin and anemia may be influenced by the presence of inflammation, we performed separate analyses for participants with normal CRP levels and participants with elevated CRP levels. The association between low ferritin and anemia was evident in participants with elevated CRP levels (n=171). In this subgroup, low ferritin was associated with a 7-fold increased risk of anemia (OR 7.0 [95% CI 1.4;34.9]). No significant association was found between low ferritin and anemia in participants with normal CRP levels (n=341, OR 1.7 [95% CI 0.7;4.2]).

To further explore these findings, we performed additional analyses in which men and women were divided separately into four groups based on ferritin status and inflammatory status. For both genders, mean hemoglobin levels differed significantly between the four groups (Figure 1, one-way ANOVA, p<0.001). Lowest hemoglobin levels were found in participants with low ferritin levels and elevated CRP levels: mean hemoglobin level 99 g/L (standard error [SE] 11) in men and mean hemoglobin level 112 g/L (SE 8) in women. Similar results were found for MCV (Figure 2). Lowest MCV values were found for men and women with low ferritin levels and elevated CRP levels (mean MCV 78 fl [SE 6] in men and mean MCV 84 fl [SE 1] in women).

During 5 years of follow-up, 166 participants developed anemia (mean follow-up time 3.7 years [95% CI 3.5-3.8]). Participants with low ferritin levels had an increased risk of developing anemia over time compared to those with normal ferritin levels (hazard ratio [HR] 1.7 [95% CI 1.0-2.9]). Similar hazard ratios were
found when we stratified on the presence of inflammation (in participants with normal CRP levels: HR 1.7 [95% CI 0.9-3.2], in participants with elevated CRP levels: HR 2.0 [95% CI 0.8-5.0]).

With linear regression analyses we estimated the effect of low ferritin levels at age 85 years on changes in hemoglobin levels and MCV during follow-up (Table 3). In the total study population, low ferritin was associated with an additional decline in hemoglobin level (additional annual change in hemoglobin -2 g/L [95% CI -4;-0.3]) and MCV (additional annual change in MCV -0.78 fL [95% CI -1.3; -0.22]) in the years thereafter.
Again, these associations were most apparent in participants with elevated CRP levels. In this subgroup, low ferritin was associated with an additional annual decline in hemoglobin level of 5 g/L (additional annual change in hemoglobin level -5 g/L [95% CI -10;-0.4]) and an additional annual decline in MCV of 2.2 fL (additional annual change in MCV -2.2 fL [95% CI -3.6;-0.71]).

### Table 3. Changes in hemoglobin and MCV during follow-up depending on low ferritin levels* at age 85 years

<table>
<thead>
<tr>
<th>Nlow ferritin</th>
<th>Hemoglobin</th>
<th>MCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population</td>
<td>35/512</td>
<td>-2 (-4;0.3)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>26/341</td>
<td>-2 (-4;1)</td>
</tr>
<tr>
<td>Elevated†</td>
<td>9/171</td>
<td>-5 (-10;0.4)</td>
</tr>
</tbody>
</table>

MVC = mean corpuscular volume.

Additional annual changes were estimated with linear regression analysis. All models were adjusted for gender.

* ferritin <20 µg/L for men and <15 µg/L for women
† C-reactive protein >5 mg/L

**DISCUSSION**

The present study shows that low ferritin levels in older individuals in the general population are associated with the prevalence of anemia and with a larger decline in hemoglobin level and MCV during follow-up. This association was most prominent in participants with signs of an inflammatory host response in plasma.

The diagnostic value of serum ferritin levels to detect iron deficiency in patients with anemia, infections and inflammation has been questioned by others because of ferritin’s ‘acute phase’ properties. Serum ferritin may be falsely high during acute and chronic inflammatory conditions and therefore not be an accurate reflection of true iron status. However, the findings that we have presented here show the significance of measuring ferritin levels in older individuals, especially among those with infections or inflammation, because a low ferritin level identifies those at risk of having or developing anemia. This is in line with the results of a large meta-analysis by Guyatt and colleagues in which highest post-test probabilities for iron deficiency for any given ferritin cut-off value were found in populations with inflammatory disease. In these patients, a low ferritin is a specific marker of iron status due to its ‘acute phase’ properties: iron status must be poor when low ferritin levels are found in the presence of systemic inflammation.

Ferritin is an acute phase protein and is elevated during acute and chronic inflammatory conditions. In our study, the association between low ferritin levels...
Ferritin, anemia and inflammation

and anemia was most apparent in participants with high CRP levels. How can we explain the occurrence of low ferritin levels in the presence of inflammation?

First, some older patients with gastrointestinal tumours or chronic inflammatory diseases (such as heart failure or end stage renal disease) will have decreased iron stores, either due to gastrointestinal blood loss, malnutrition, or malabsorption of food-bound iron. In these individuals, iron stores may have become too low to facilitate a rise in ferritin in response to inflammation.

Second, upregulation of hepcidin – the main regulator of iron homeostasis – is an alternative molecular pathway to explain our findings. An inflammatory stimulus activates monocytes and T cells to produce pro-inflammatory cytokines. These cytokines, particularly interleukin 6, induce the production and secretion of hepcidin by hepatocytes. Hepcidin binds to the membrane protein ferroportin, an iron efflux channel on the surface of absorptive enterocytes, macrophages and hepatocytes, and induces its internalization and degradation in lysosomes, thereby blocking the export of iron from cells. Consequently, duodenal enterocytes deliver less dietary iron to extracellular fluid, macrophages fail to release iron recycled from senescent erythrocytes and hepatocytes retain stored iron, leading to a rapid drop in iron levels, iron-restricted erythropoiesis, and anemia. Moreover, transgenic mice overexpressing hepcidin and mice receiving synthetic hepcidin develop mild-to-moderate microcytic, hypochromic anemia. As a result, hepcidin is considered to be the main mediator of anemia of inflammation, also known as anemia of chronic disease, which is commonly found in patients with chronic infections or with inflammatory disorders, such as rheumatoid arthritis, inflammatory bowel disease, cancer and chronic kidney disease. We now hypothesize that chronic inflammation induces a persistent elevation in hepcidin that eventually leads to a severe decline in iron stores and, as a result, a severe decline in serum ferritin level that “overrules” the initial acute rise in ferritin level in response to inflammation. Although a preliminary analysis in the InChianti study, a population-based study of older persons in Tuscany, Italy, could not demonstrate higher urinary hepcidin levels in older individuals with anemia of inflammation, we suggest this hypothesis to be tested in other population-based studies, preferably using serum hepcidin assays, which recently have become available. Depending on the outcomes of these additional studies, future diagnostic algorithms for anemia may incorporate markers of inflammation such as CRP or even hepcidin to discriminate between classic iron deficiency anemia (low hepcidin levels) and iron deficiency anemia in the context of anemia of chronic disease (elevated hepcidin levels).

A strong point of our study is its population-based setting with almost complete follow-up, permitting us to generalize our conclusions to older people in the general population at large. Another strength is its prospective design, allowing us...
to investigate the effect of low ferritin levels on subsequent changes in hemoglobin levels and MCV over time.

It is a limitation of our study that we did not have other measurements of iron status, such as serum iron and total iron binding capacity, which are usually included in algorithms for the evaluation of anemia.\textsuperscript{4,9,10} As a result, we cannot rule out that some participants with normal or high ferritin levels in fact had iron deficiency. This may have led to an underestimation of the effect of low ferritin on hemoglobin and MCV, especially in participants with inflammation. Another limitation of our study is the lack of hepcidin measurements which inhibited us to further explore some of the underlying pathophysiological mechanisms that could explain our findings.

The relatively small sample size of our study, especially of participants with low ferritin levels and elevated CRP levels, may also be considered a potential limitation of our study. In the Cox proportional hazard analyses, we did not have sufficient power to show a statistically significant effect of low ferritin levels on the development of anemia in participants with and without inflammation. Yet, the results of the Cox proportional hazard models indicated a stronger association between low ferritin and the development of anemia in participants with elevated CRP levels compared to participants with normal CRP levels. This was confirmed in the linear regression analyses in which we, even within the small group of participants with elevated CRP levels, still found a statistically significant association between low ferritin levels and the change in hemoglobin level and MCV over time.

In the present study, we explored the association between low ferritin levels and anemia in participants with and without inflammation. Since our study is an etiologic study, and neither a diagnostic study nor an intervention study, the results of our study do not yet have direct implications for clinical practice with respect to the diagnosis and treatment of anemia in old age. Still, the results of our study add to the body of evidence that some important issues about the diagnosis and treatment of anemia in old age should be investigated further.

In conclusion, the present study shows that low ferritin levels in older individuals are still associated with anemia, especially when inflammation is present. Future studies are needed to gain more insight into the pathophysiologic mechanisms and clinical implications of these findings.

**FUNDING**

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REFERENCES


