Strategies for the Laboratory Diagnosis of Some Common Causes of Anaemia in Elderly Patients

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Key Words
Anaemia · Elderly, anaemia · Diagnostic criteria, anaemia

Abstract
Anaemia is a common medical problem in elderly patients and is associated with an increased mortality and morbidity risk and a reduced quality of life. It is not known at which exact haemoglobin level investigations should be initiated in order to optimize the diagnostic efficacy. Serum ferritin determination remains the most accurate laboratory test for the diagnosis of iron deficiency anaemia and its differential diagnosis with the anaemia of chronic disease. The introduction of the metabolites methylmalonic acid and homocysteine has made it possible to diagnose vitamin B12 and folate deficiencies at an early subclinical stage, even without neurological and haematological symptoms, but the clinical importance of this ‘biochemical’ diagnosis is unclear. Other causes of anaemia, such as myelodysplastic syndromes and chronic renal insufficiency, will become more and more common in the elderly because of the ageing of the population. Although erythropoietin analysis has no clear diagnostic value at the moment, it has become more and more obvious that its therapeutic importance in elderly patients with chronic anaemia is increasing. A substantial number of patients have an unexplained anaemia. Whether this is disease related, or may be attributed to an age-related anaemia, is still a matter of debate, but it is advisable to perform an extensive laboratory, cytogenetic, and morphological investigation before one should assess the anaemia as unexplained.

Introduction
Anaemia is a common problem in elderly patients and is associated with an increased mortality and morbidity risk and a reduced quality of life [1, 2]. It may result from a decreased red blood cell production, an increased red blood cell turnover, or red blood cell loss from the circulation by bleeding. In clinical practice, anaemia is defined as a haemoglobin value below a specific decision limit. According to the World Health Organization (WHO), anaemia is defined as a haemoglobin value below a specific decision limit. This decision limit is mainly chosen arbitrarily or is the result of a statistical consideration (mean value minus 2 SD in a ‘healthy’ population). According to the World Health Organization (WHO), anaemia is defined as a haemoglobin level <13 g/dl for men and <12 g/dl for women.
In a recent study [3], the WHO criteria were compared to age-specific decision limits for haemoglobin. The epidemiological decision limits for anaemia in a healthy elderly group between 70 and 88 years of age declined for men from 12.8 to 11.6 g/dl and for women from 11.8 to 11.4 g/dl [3]. The age-associated prevalence of anaemia, thus defined, is relatively stable (3.2–9.7%), whereas 28.3% of the 88-year-old men and 9.3% of the 88-year-old women in the same study had anaemia according to the WHO criteria [3]. These criteria overlook the clinical and functional conditions of the patients, and the ideal haemoglobin level is that level with the lowest mortality and morbidity rate and the highest quality of life.

There are some reasons why mild anaemia should not be considered ‘physiological’ in elderly patients. A haemoglobin level of 13.5 g/dl was associated with a significantly lower mobility difficulty prevalence than a haemoglobin concentration of 12 g/dl in elderly women [4]. The 30-day mortality in elderly patients with acute myocardial infarction is nearly double in those with a haematocrit <24% compared with those with a haematocrit >36%, and blood transfusion improved the prognosis [5]. The quality of life correlates with the increase in haemoglobin in cancer patients treated with erythropoietin, with the greatest improvement for each 1-g/dl change in haemoglobin occurring when the haemoglobin level increased from 11 to 12 g/dl [6]. It is not known at what exact haemoglobin level investigations should be initiated in order to optimize the diagnostic efficacy.

In an elderly anaemic population, non-haematological causes such as chronic inflammation and infection, cancer, gastro-intestinal lesions, and chronic renal insufficiency are more frequent than the classical haematological disorders such as leukaemia, multiple myeloma, and lymphomas.

The aim of this review is to summarize the clinical usefulness of the diagnostic criteria for the most important causes of anaemia in elderly patients, of which the anaemia of chronic disease (ACD) and iron deficiency anaemia (IDA) account for more than half in elderly hospitalized and community-based elderly (table 1) [7, 8].

### Anaemia of Chronic Disease

This hypoproliferative anaemia was originally described as the anaemia of infection and later as ACD, although there is some debate about its nomenclature [9, 10]. It is the most common form of anaemia in a hospitalized geriatric population and is defined as the anaemia associated with chronic inflammatory disorders, chronic infections, and malignancy. A common characteristic is a low serum iron concentration despite adequate reticuloendothelial iron stores. However, other than these classical disorders, heart failure, myocardial infarction, and thrombophlebitis must also be considered as causes of ACD [11]. The prototype of ACD is rheumatoid arthritis, but in elderly patients, a heterogeneous group of infectious, inflammatory, and neoplastic diseases, each with a specific underlying pathogenetic mechanism, are more frequently the underlying cause of the ACD. An impaired erythropoietin-dependent erythropoiesis triggered by pro-inflammatory cytokines, impaired iron mobilization, and shortened red cell survival is involved in the pathogenesis of ACD [12].

The diagnosis of ACD is typically made on the basis of a low serum iron concentration, a low total iron-binding capacity, a low serum transferrin concentration, a low iron saturation index, and a normal or elevated serum ferritin level together with the presence of a chronic disease.
For none of these laboratory parameters standard reference diagnostic criteria are available. Table 2 shows the prevalence of some laboratory parameters according to arbitrarily chosen decision limits in 72 elderly hospitalized patients: 34 patients with IDA and 38 patients with ACD [13]. A typical patient with ACD has a moderate (haemoglobin level between 8 and 11 g/dl) and normocytic (MCV or mean corpuscular volume between 80 and 100 fl) anaemia, but the anaemia may be more severe and microcytic (MCV <80 fl) in a substantial number of patients (table 2). The clinical importance of the serum transferrin receptor analysis for the differential diagnosis between ACD and IDA will be discussed below.

Some additional markers such as erythrocyte sedimentation rate, C-reactive protein, and zinc protoporphyrin are sometimes used as additional parameters in order to emphasize the inflammatory character of the anaemia, but their additional diagnostic importance is unclear.

Iron Deficiency Anaemia

Iron is essential for carriage of oxygen by haemoglobin, for oxidative metabolism, and for normal cellular growth. Plasma transferrin binds two iron atoms and carries iron to the transferrin receptor. This transferrin receptor-transferrin complex is internalized, and iron is released and accumulates within the cells in the form of ferritin, while the complex returns to the cell surface [14].

The standard diagnostic test for iron deficiency is iron staining of a bone marrow aspirate with absent iron stores. From a clinical point of view, two subgroups are important: patients with absent iron stores and normal haemoglobin levels (iron deficiency) and patients with absent iron stores and anaemia (IDA). The prevalence of iron deficiency varies according to the diagnostic criteria used. The National Health and Nutrition Examination Survey (NHANES III) study showed that 7% of the women and 4% of the men older than 70 years had iron deficiency, while 2% of both men and women had IDA [15].

For many years, the classical biochemical markers used for the diagnosis of iron deficiency have been serum iron and transferrin, iron saturation index, MCV, and serum ferritin. There is an overlap for these laboratory parameters between ACD and IDA (table 3), and the differential diagnosis between both disorders remains a clinical challenge. The majority of the patients in both groups have a MCV >80 fl and low serum iron levels which makes both tests of limited importance for the differential diagnosis between ACD and IDA. However, a MCV <70 fl is rarely seen in patients with ACD. The serum ferritin level is the most powerful laboratory parameter for the diagnosis of absent iron stores [16–18]. A serum ferritin level below the lower reference limit indicates absent iron stores, but does not inform about its severity. However, the clinical interpretation of the test result may be complicated by the fact that serum ferritin is an acute-phase reactant; the levels increase with age, and the lower reference limit is not well defined. In the literature, this lower reference limit varies between 12 and 100 µg/l, depending on sex, age, and patient selection. A serum ferritin level of 12 µg/l is mostly used from a historical point of view [19]. This value has a high specificity, but an unacceptably low sensitivity, because most elderly patients with IDA have a serum ferritin level >12 µg/l. In a previous study [17], we found a lower reference limit of 50 µg/l for serum ferritin, the best discriminant between IDA and non-IDA in an elderly population. This is in agreement with the guidelines published in a recent study, where iron deficiency was defined in men and postmenopausal women as a serum ferritin level <45 µg/l [20].

It has been suggested that a new test, the immunoassay of soluble transferrin receptor (sTfR) in serum, is useful for distinguishing IDA from ACD in a non-invasive way [21–24]. The transferrin receptor is a transmembrane glycoprotein that is expressed on most cells, especially on those that require high iron levels. A major benefit of sTfR over ferritin may be that it is not an acute-phase reactant. The sTfR level and the ratio of sTfR to the log serum ferritin level have been shown to be promising parameters to distinguish between IDA, IDA with concurrent chronic inflammation or infection, and ACD. Results of clinical studies assessing the effectiveness of the sTfR in elderly patients are contradictory [13, 22, 25, 26], and data about the influence of age on the sTfR levels are not available. A low-dose oral iron absorption test has been

### Table 3. Differential diagnosis between ACD and IDA in the elderly (values used arbitrarily chosen)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IDA</th>
<th>ACD</th>
</tr>
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<tbody>
<tr>
<td>MCV, fl</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Serum ferritin, µg/l</td>
<td>&lt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Serum iron, µg/dl</td>
<td>&lt;70</td>
<td>&lt;70</td>
</tr>
<tr>
<td>Saturation index, %</td>
<td>&lt;25</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Iron-staining bone marrow aspirate</td>
<td>absent</td>
<td>normal or increased</td>
</tr>
<tr>
<td>Serum transferrin receptor</td>
<td>diagnostic significance unclear</td>
<td></td>
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</table>
used as a simple test to distinguish patients with and without iron deficiency [27, 28]. However, there are no standardized criteria for this test, and data on its clinical usefulness are limited.

It remains important to emphasize that iron deficiency with or without anaemia is just a symptom of an underlying disease and is considered a sign of chronic blood loss until proven otherwise. Therefore, a thorough gastrointestinal exploration is the next step for further investigation [29]. The introduction of a simple guideline diagnosis of iron deficiency if serum ferritin <45 µg/l led to a significant increase in the proportion of patients with IDA and in the detection of serious gastro-intestinal lesions [20].

**Folate and Vitamin B\(_{12}\) Deficiency Anaemia**

Vitamin B\(_{12}\) and folate deficiencies are typically characterized by low erythrocyte and serum folate levels or a low serum vitamin B\(_{12}\) level with a macrocytic anaemia, a megaloblastic bone marrow, and neuropsychiatric symptoms. This clinical picture with pernicious anaemia as the classical example has become less and less common. At the other end of the spectrum, macrocytosis and anaemia are absent, the underlying causes of vitamin B\(_{12}\) or folate deficiency are less obvious or even unknown, and the clinical picture is usually atypical or absent. This deficiency state is often labelled as mild or subclinical and is mainly defined by elevated serum metabolite levels of methylmalonic acid (MMA) and/or total homocysteine (tHcy). There are no gold standards for the diagnosis of vitamin B\(_{12}\) and folate deficiencies, and there remains a lot of controversy regarding the best interpretation of the different assays.

Serum vitamin B\(_{12}\) and folate and erythrocyte folate are widely available and most commonly used to assess the vitamin status. The lower reference limits are variable: usually between 170 and 200 ng/l for serum vitamin B\(_{12}\), around 3 µg/l for serum folate, and between 150 and 200 µg/l for erythrocyte folate. The red cell folate assay may be a more accurate test to evaluate the folate stores, because it is not influenced by the dietary intake, but its concentration is affected by the vitamin B\(_{12}\) status and the high variation coefficient of the assay [30, 31]. Published estimates for the sensitivity and specificity of these tests in the diagnosis of vitamin B\(_{12}\) and folate deficiencies vary widely, and this is partly related to the criteria used to define the vitamin deficiency. False low serum vitamin B\(_{12}\) levels occur commonly in folate deficiency (table 4) and pregnancy and in myelomatosis and haptocorrin deficiency; false normal or increased vitamin B\(_{12}\) levels are present in myeloproliferative disorders, liver diseases, after nitrous oxide anaesthesia, and in transcobalamin deficiency [32–34]. A false normal or increased serum folate level may occur occasionally in vitamin B\(_{12}\) deficiency (table 4); a low erythrocyte folate level without deficiency is common in vitamin B\(_{12}\) deficiency, and a normal level in the presence of deficiency can occur with acute folate deficiency [32–34].

During the last decade, the introduction of the metabolite assays, MMA and homocysteine, has made it possible to diagnose vitamin B\(_{12}\) and folate deficiencies at an earlier and subclinical stage [35, 36]. MMA is a specific marker for vitamin B\(_{12}\) deficiency, while the tHcy level is also increased in folate and vitamin B\(_{6}\) deficiencies (table 4). Especially the tHcy level has become interesting, because an elevated level is associated with an increased risk of atherosclerotic diseases [37]. There are no standard diagnostic criteria for MMA and tHcy levels, and the levels of both metabolites are influenced by renal function, age, and sex [38, 39]. Some authors have suggested that the combination of a higher than normally used vitamin level with an elevated metabolite level (e.g., serum vitamin B\(_{12}\) level <300 ng/l and serum MMA level >271 nmol/l) is a more accurate way to diagnose the vitamin deficiency. Whether this ‘biochemical’ approach facilitates the diagnosis of clinically significant vitamin deficiencies remains unclear.

In a study performed by Savage et al. [40], 98.4 and 95.9% of the patients with vitamin B\(_{12}\) deficiency had elevated serum MMA and tHcy levels, respectively, and 91% of the patients with folate deficiency had elevated tHcy levels. In this study, the diagnosis of vitamin B\(_{12}\) and folate deficiencies was based on serum vitamin levels,
Criteria of a megaloblastic haematopoiesis [44]. In a recent study [44], patients older than 70 years and with a MCV level increases to 110 fl or the slightly elevated MVC is accompanied by other pathological findings such as anaemia with a low reticulocyte count, low serum vitamin levels, leucopenia, and thrombocytopenia. In a recent study [44], patients older than 70 years and with a MCV >100 fl were investigated. Only 15% had morphological criteria of a megaloblastic haematopoiesis [44]. In plasma, the majority of vitamin B12 is bound to two carrier proteins, transcobalamin and haptocorrin. A decreased saturated (holo)transcobalamin level could be the earliest marker of vitamin B12 deficiency, but this analysis has not been clinically validated as yet, and the clinical results are disappointing [45–47]. Examination of a peripheral blood smear remains an important non-invasive diagnostic tool, but is performed less often, and a bone marrow aspirate is useful, especially when the diagnosis is in doubt (especially for the differential diagnosis with the myelodysplastic syndromes – MDS).

In a second stage, additional investigations in vitamin B12 deficient patients, including the measurement of intrinsic factor and parietal cell antibodies, malabsorption tests such as the classical Schilling test and the protein-bound vitamin B12 absorption test, upper gastro-intestinal endoscopy, and a careful dietary investigation, may be performed in order to find a proper cause for the vitamin B12 deficiency. The main cause of folate deficiency is dietary related. An extensive discussion of these diagnostic procedures is outside the scope of this article.

Myelodysplastic Syndromes

MDS are a morphologically and clinically heterogeneous group of acquired stem cell disorders characterized by ineffective and dysplastic haematopoiesis in one or more cell lines that affect predominantly older subjects [48]. The patients usually present with anaemia and dysplastic morphologic abnormalities in cells of more than one lineage (neutropenia, thrombocytopenia, monocytosis, or a combination of types of cytopenias). The diagnosis is essentially based on the morphological examination of a blood smear and a bone marrow biopsy specimen. The French-American-British classification divides the MDS into five subtypes based on the number of immature blast cells and morphological abnormalities in blood and bone marrow and the presence of ringed sideroblasts [49]. The WHO classification of MDS is an extension of the French-American-British classification that includes cytogenetic data. The survival in patients with MDS varies widely, ranging from weeks to years, and depends on factors such as age, cytogenetic abnormalities, percentage of blasts, and number of haematopoietic lineages involved in the cytopenia [50]. The extensive diagnostic workup and treatment belongs to the field of haematology, although therapy is essentially supportive in elderly patients.

Other Haematological and Non-Haematological Disorders

Haemolytic anaemia, aplastic anaemia, lymphoproliferative disorders, and paraproteinaemia occasionally occur in an elderly population as the underlying causes of anaemia [51]. These disorders need a specialistic diagnostic and therapeutic approach. Numerous non-haematological systemic disorders affect the blood in different ways, and anaemia is the most common haematological abnormality: acute and chronic renal failure, endocrine diseases such as hyper- and hypothyroid disease, adrenal and pituitary failure, and liver diseases such as viral hepatitis and cirrhosis [52]. The pathogenesis is multifactorial, the anaemia is mostly diagnosed in the presence of the primary disease, but the exact diagnosis may be difficult when the underlying systemic disease progresses insidiously. An in-depth diagnostic strategy for these disorders is outside the scope of this article.

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Gerontology 2004;50:49–56

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Algorithm for the evaluation of anaemia in elderly patients

History (drugs, alcohol, melena, etc.) and physical examination (hepatosplenomegaly, adenopathy, icterus, etc.) are directives for further investigation

Normal or decreased absolute reticulocyte count

<table>
<thead>
<tr>
<th>MCV ≥ 100 fl</th>
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<tr>
<td>-Check serum vitamin B₁₂ and erythrocyte and serum folate and MMA and thy if available vitamin B₁₂ or folate deficiency</td>
</tr>
<tr>
<td>-Consider</td>
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<tr>
<td>-Alcoholism</td>
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<tr>
<td>-Liver disease</td>
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<tr>
<td>-Marrow disorders: e.g., MDS, leukaemia, etc.</td>
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Increased absolute reticulocyte count

<table>
<thead>
<tr>
<th>Acute blood loss</th>
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<tr>
<td>Haemolytic anaemia</td>
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MCV < 100 fl

-Consider chronic renal failure
-Check serum ferritin
-Serum ferritin < 50 μg/l IDA
- Serum ferritin > 100 μg/l, low serum iron, and low saturation index ACD

Consider bone marrow investigation if serum ferritin between 60 and 100 μg/l (diagnostic diagnosis between IDA or ACD) or if unknown cause after clinical and laboratory investigation (e.g., to exclude MOS)

Fig. 1. Algorithm for the evaluation of anaemia in elderly patients. Notes: An MCV level < 80 fl does not exclude the diagnosis of ACD. Most patients with IDA and vitamin B₁₂ and folate deficiencies have MCV values of between 80 and 100 fl. A low serum iron level alone is not useful as a criterion for the diagnosis of IDA. One must always consider the possibility of more than one underlying cause for the anaemia.

Anaemia of Unknown Origin

In 17% of our patients admitted to the acute geriatric ward, no underlying cause for the anaemia was found (table 1). This percentage is even higher in non-hospitalized elderly patients [8]. This can be explained, at least partly, by an incomplete diagnostic evaluation by which some common causes such as ACD, iron deficiency anaemia, or MDS were not identified. However, one could hypothesize that some age-related changes in the erythropoiesis may be responsible for this ‘age-related’ anaemia. A reduced erythropoietin level seems to play a role in the pathogenesis of unexplained anaemia in the elderly [53], but studies comparing the erythropoietin and haemoglobin levels between elderly and younger controls with different types of anaemia provide conflicting results [54]. The role of a dysregulation in pro-inflammatory cytokines such as interleukin-6 in the pathogenesis of an age-associated anaemia needs further investigation [55]. Data on the erythropoietic precursors in the bone marrow are not conclusive. In 1984, Lipschitz et al. [56] demonstrated that healthy elderly males with unexplained anaemia had significantly lower bone marrow erythroid precursor levels than the young or elderly controls. Progenitor cell abnormalities do not seem to play a role in elderly patients with unexplained anaemia, but elderly men showed lower values for erythroid progenitors than women [57]. However, there is a trend toward a decreased absolute number of CD34+ (early multipotent precursor) progenitor cells in the peripheral blood in older adults and centenarians, but the capability of these cells to respond to haemopoietic cytokines and to form erythroid progenitor cells is well preserved [58]. In conclusion, a substantial number of elderly patients have an unexplained anaemia after extensive laboratory, cytogenetic, and morphological investigations. Whether this is disease related or may be attributed to an age-related anaemia is still a matter of debate.
Diagnosis of Anaemia in the Elderly

Concluding Remarks

Anaemia remains a frequent clinical problem in elderly patients. It is not known at which exact haemoglobin level investigations should be initiated in order to optimize the diagnostic efficacy. However, there is no clear reason why mild anaemia should be less indicative of important diseases than severe anaemia.

Despite its role as an acute-phase reactant, determination of the serum ferritin concentration remains the most accurate laboratory test for the diagnosis of IDA and for the differential diagnosis between IDA and ACD. Elderly patients with a serum ferritin level <50 μg/l have a very high probability of iron deficiency. The role of the serum transferrin receptor level and the serum transferrin receptor-log serum ferritin ratio is still unclear. The introduction of the metabolites MMA and tHcy has made it possible to diagnose vitamin B12 and folate deficiencies at an early subclinical stage, even in the absence of neurological and haematological symptoms, but the clinical importance of this ‘biochemical’ diagnosis is unclear. These tests are not widely available, and the clinical benefit of vitamin B12 supplementation in patients with subclinical B12 deficiency (moderately elevated MMA levels) is a matter of debate. Whether homocysteine-lowering therapy may be beneficial in patients with atherosclerotic disorders will finally be answered by several ongoing intervention trials. Other causes of anaemia, such as MDS and chronic renal insufficiency, will become more and more common in the elderly because of the ageing of the population. Although erythropoietin analysis has no clear diagnostic value at the moment, it has become more and more obvious that its therapeutic importance in elderly patients with chronic anaemia is increasing. An algorithm for the evaluation of the anaemic elderly patient is shown in figure 1. The aim of such an algorithm is not to seek completeness, but to present practical guidelines. The investigation can be hampered by the fact that many elderly patients may have more than one cause for their anaemia. Finally, a substantial number of patients have an unexplained anaemia after extensive laboratory, cyto genetic, and morphological investigations. Whether this is disease related or may be attributed to an age-related anaemia is still a matter of debate.

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