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

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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 B_{12} 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. 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.

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Table 1. Different causes of anaemia (defined as a haemoglobin level <11.5 g/dl) in 178 hospitalized elderly patients [from ref. 7]

	%
ACD	35
IDA	15
Posthaemorrhagic anaemia	7
Renal failure and liver and endocrine diseases	6.5
MDS and acute leukaemia	5.5
Lymphoma-related anaemia	5.5
Vitamin B_{12} or folate deficiency	5.5
Other haematological disorders	3
Unknown	17

Table 2. Prevalence distribution of some laboratory parameters according to arbitrarily chosen decision limits in patients with ACD and IDA [from ref. 11]

	IDA, % (n = 34)	ACD, % (n = 38)
C-reactive protein >5 mg/l	79	95
C-reactive protein >10 mg/l	53	87
Erythrocytic sedimentation rate >30 mm/h	65	85
MCV >80 fl	74	87
Haemoglobin <8 g/dl	9	8
Serum iron <60 µg/dl	97	90
Saturation index <25%	100	87

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 proinflammatory 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

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

	IDA	ACD
MCV, fl	<100	<100
Serum ferritin, µg/l	<50	>50
Serum iron, µg/dl	<70	<70
Saturation index, %	<25	<25
Iron-staining bone marrow aspirate	absent	normal or increased
Serum transferrin receptor	diagnostic significance unclear	

<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 4. Performance of serum vitamin and metabolite levels in patients with vitamin B_{12} and folate deficiencies

	Vitamin B_{12} deficiency	Folate deficiency	Vitamin B_{12} deficiency + folate deficiency
Serum vitamin B_{12}	↓	normal or ↓	↓
Serum folate	normal or ↑	↓	↓
Erythrocyte folate	normal or ↓	↓	↓
Serum MMA	↑	normal	↑
Serum tHcy	↑	↑	↑

↑ = Elevated level; ↓ = decreased level.

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,

haematological and neurological findings, and response to therapy. The prevalence of elevated metabolite levels in elderly patients is much higher than the prevalence of low serum vitamin B_{12} and folate levels [41]. However, of the 419 patients with clinical vitamin B_{12} deficiency, only 2.9% were identified as being vitamin B_{12} deficient despite the presence of a serum vitamin B_{12} level >200 ng/l [42]. It remains a crucial problem to what extent elevated metabolite levels in those patients without haematological and clinical symptoms (subclinical deficiency) are of clinical importance or whether they need vitamin supplementation. In a recently published study [43], it was demonstrated that the clinical benefit of vitamin B_{12} treatment in patients with a mild to moderate increase in serum MMA (up to 600 nmol/l) was limited, irrespective of the serum vitamin levels. On the other hand, the clinical significance of tHcy is different. Many studies have demonstrated that an elevated homocysteine level is an increased risk factor for the development of atherosclerotic diseases, but at present no data from intervention trials are available.

A slightly elevated MCV as the only test for screening is of limited use, but gains some importance when the MCV 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 B_{12} is bound to two carrier proteins, transcobalamin and haptocorrin. A decreased saturated (holo)transcobalamin level could be the earliest marker of vitamin B_{12} 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 B_{12} 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 B_{12} absorption test, upper gastro-intestinal endoscopy, and a careful dietary investigation, may be performed in order to find a proper cause for the vitamin B_{12} 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.
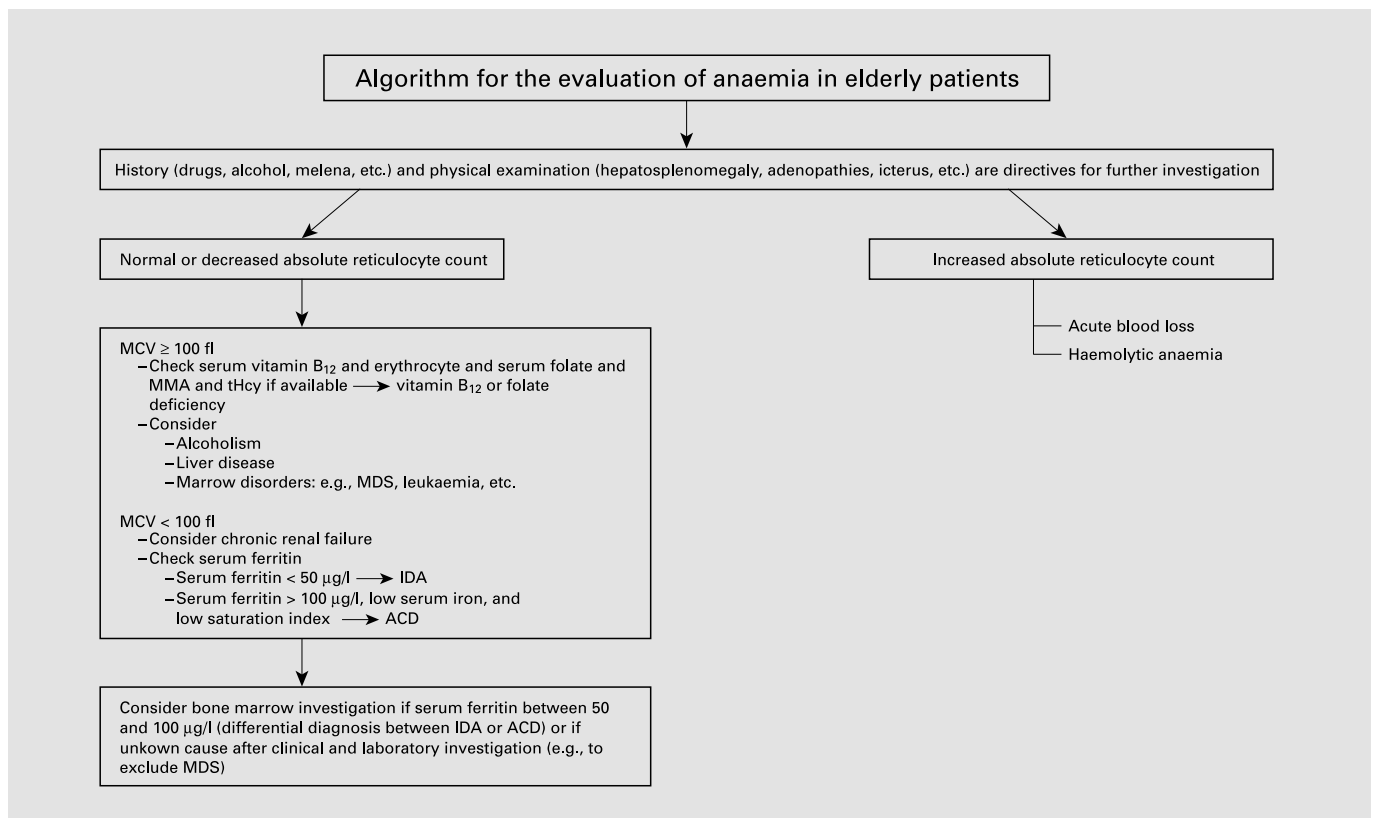
Algorithm for the evaluation of anaemia in elderly patients

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

Normal or decreased absolute reticulocyte count

Increased absolute reticulocyte count
— Acute blood loss
— Haemolytic anaemia

MCV ≥ 100 fl
  — Check serum vitamin B_{12} and erythrocyte and serum folate and MMA and tHcy if available ⟶ vitamin B_{12} or folate deficiency
  — Consider
      — Alcoholism
      — Liver disease
      — Marrow disorders: e.g., MDS, leukaemia, etc.

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 50 and 100 µg/l (differential diagnosis between IDA or ACD) or if unkown cause after clinical and laboratory investigation (e.g., to exclude MDS)

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 B_{12} 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 unexplaind 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.

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 B_{12} 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 B_{12} supplementation in patients with subclinical B_{12} 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, cytogenetic, and morphological investigations. Whether this is disease related or may be attributed to an age-related anaemia is still a matter of debate.

References

1 Izaks GJ, Westendorp RG, Knook DL: The definition of anemia in older persons. JAMA 1999;281:1714–1717.
2 Lipschitz D: Medical and functional consequences of anemia in the elderly. J Am Geriatr Soc 2003;51(suppl):10–13.
3 Nilsson-Ehle H, Jagenburg R, Landahl S, Svanborg A: Blood haemoglobin declines in the elderly: Implications for reference intervals from age 70 to 88. Eur J Haematol 2000;65:297–305.
4 Chaves PH, Ashar B, Guralnik JM, Fried LP: Looking at the relationship between hemoglobin concentration and prevalent mobility difficulty in older women: Should the criteria currently used to define anemia in older people be re-evaluated? JAMA 2002;50:1257–1264.
5 Wu WC, Rathore SS, Wang Y, Radford M, Krumholz HM: Blood transfusion in elderly patients with acute myocardial infarction. N Engl J Med 2001;345:1230–1236.
6 Littlewood TJ, Bajetta E, Nortier JW, Vercammen E, Rapoport B: Effects of epoetin alfa on hematologic parameters and quality of life in cancer patients receiving nonplatinum chemotherapy: Results of a randomized, double-blind, placebo-controlled trial. J Clin Oncol 2001;19:2865–2874.
7 Joosten E, Pelemans W, Hiele M, Noyen J, Verhaeghe R, Boogaerts MA: Prevalence and causes of anaemia in a geriatric hospitalised population. Gerontology 1992;38:111–117.

8 Ania BJ, Suman VJ, Fairbanks VF, Rademacher DM, Melton LJ 3rd: Incidence of anemia in older people: An epidemiologic study in a well-defined population. J Am Geriatr Soc 1997;45:825–831.
9 Lee GR: The anemia of chronic disease. Semin Hematol 1983;20:61–80.
10 Schilling RF: Anemia of chronic disease: A misnomer. Ann Intern Med 1991;115:572–573.
11 Cash JM, Sears DA: The anemia of chronic disease: Spectrum of associated diseases in a series of unselected hospitalized patients. Am J Med 1989;87:638–644.
12 Means RT: The anaemia of infection. Baillières Clin Haematol 2000;13:151–162.
13 Joosten E, Van Loon R, Billen J, Blanckaert N, Fabri R, Pelemans W: Serum transferrin receptor in the evaluation of the iron status in elderly hospitalized patients with anemia. Am J Hematol 2002;69:1–6.
14 Andrews NC: Disorders of iron metabolism. N Engl J Med 1999;341:1986–1995.
15 Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL: Prevalence of iron deficiency in the United States. JAMA 1997;277:973–976.
16 Guyatt GH, Patterson C, Ali M, Singer J, Levine M, Turpie I, Meyer R: Diagnosis of iron deficiency anemia in the elderly. Am J Med 1990;88:205–209.

17 Joosten E, Hiele M, Ghoos Y, Pelemans W, Boogaerts MA: Diagnosis of iron deficiency anemia in a hospitalized geriatric population. Am J Med 1991;90:653–654.
18 Burns ER, Goldberg SN, Lawrence C, Wenz B: Clinical utility of serum tests for iron deficiency in hospitalized patients. Am J Clin Pathol 1990;93:240–245.
19 Lipschitz DA, Cook JD, Finch CA: A clinical evaluation of serum ferritin as an index of iron stores. N Engl J Med 1974;209:1213–1216.
20 Ioannou GN, Spector J, Scott K, Rockey D: Prospective evaluation of a clinical guideline for the diagnosis and management of iron deficiency anemia. Am J Med 2002;113:281–287.
21 Means RT Jr, Allen J, Sears DA, Schuster SJ: Serum soluble transferrin receptor and the prediction of marrow aspirate iron results in a heterogeneous group of patients. Clin Lab Haematol 1999;21:161–167.
22 Chua E, Clague JE, Sharma AK, Horan MA, Lombard M: Serum transferrin receptor assay in iron deficiency anaemia and anaemia of chronic disease in the elderly. QJM 1999;92:587–594.
23 Punnonen K, Irjala K, Rajamäki A: Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood 1997;89:1052–1057.

24 Ferguson BJ, Skikne BS, Simpson KM, Baynes RD, Cook JD: Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. J Lab Clin Med 1992; 19:385–390.

25 Rimon E, Levy S, Sapir A, Gelzer G, Peled R, Ergas D, Sthoeger ZM: Diagnosis of iron deficiency anemia in the elderly by transferrin receptor-ferritin index. Arch Intern Med 2002; 162:445–449.

26 Mast AE, Blinder MA, Gronowski AM, Chumley C, Scott MG: Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. Clin Chem 1998;44:45–51.

27 Joosten E, Vander Elst B, Billen J: Small-dose oral iron absorption test in anaemic and non-anaemic elderly hospitalised patients. Eur J Haematol 1997;58:99–103.

28 Jensen NM, Brandsborg M, Boesen AM, Yde H, Dahlerup JF: Low-dose oral iron absorption test in anaemic patients with and without iron deficiency determined by bone marrow iron content. Eur J Haematol 1999;63:103–111.

29 Joosten E, Ghesquiere B, Linthoudt H, Krekelberghs F, Dejaeger E, Boonen S, Pelemans W, Hiele M, Gevers AM: Upper and lower gastrointestinal evaluation of elderly inpatients who are iron deficient. Am J Med 1999;107: 24–29.

30 Klee GG: Cobalamin and folate evaluation: Measurement of methylmalonic acid and homocysteine vs vitamin B_{12} and folate. Clin Chem 2000;46:1277–1283.

31 Wright AJ, Finglas PM, Southon S: Erythrocyte folate analysis: A cause for concern? Clin Chem 1998;44:1886–1891.

32 Amos RJ, Dawson DW, Fish DI, Leeming RJ, Linnell JC: Guidelines on the investigation and diagnosis of cobalamin and folate deficiencies. Clin Lab Haematol 1994;16:101–115.

33 Snow CF: Laboratory diagnosis of vitamin B_{12} and folate deficiency. Arch Intern Med 1999; 159:1289–1298.

34 Zittoun J, Zittoun R: Modern clinical testing strategies in cobalamin and folate deficiency. Semin Hematol 1999;36:35–46.

35 Stabler SP, Marcell PD, Podell ER, Allen RH, Lindenbaum J: Assay of methylmalonic acid in the serum of patients with cobalamin deficiency using capillary gas chromatography-mass spectrometry. J Clin Invest 1986;77:1606–1612.

36 Stabler SP, Marcell PD, Podell ER, Allen RH, Savage DG, Lindenbaum J: Elevation of total homocysteine in the serum of patients with cobalamin or folate deficiency detected by capillary gas chromatography-mass spectrometry. J Clin Invest 1988;81:466–474.

37 Welch GN, Loscalzo J: Homocysteine and atherothrombosis. N Engl J Med 1999;338: 1042–1050.

38 Selhub J, Jacques PF, Rosenberg IH, Rogers G, Bowman BA, Gunter EW, Wright JD, Johnson CL: Serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey (1991–1994): Population reference ranges and contribution of vitamin status to high serum concentrations. Ann Intern Med 1999;131:331–339.

39 Joosten E, Lesaffre E, Riezler R: Are different reference intervals for methylmalonic acid and total homocysteine necessary in elderly people? Eur J Haematol 1996;57:222–226.

40 Savage DG, Lindenbaum J, Stables SP, Allen RH: Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiency. Am J Med 1994;96:239–246.

41 Joosten E, van den Berg A, Riezler R, Naurath HJ, Lindenbaum J, Stabler SP, Allen RH: Metabolic evidence that deficiencies of vitamin B_{12} (cobalamin), folate and vitamin B_6 occur commonly in elderly people. Am J Clin Nutr 1993; 58:468–476.

42 Lindenbaum J, Savage DG, Stabler SP, Allen RH: Diagnosis of cobalamin deficiency. II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. Am J Hematol 1990;34:99–107.

43 Hvas AM, Ellegaard J, Nexo E: Vitamin B_{12} treatment normalizes metabolic markers but has limited clinical effect: A randomized placebo-controlled study. Clin Chem 2001;47:1396–1404.

44 Savage DG, Ogundipe A, Allen RH, Stabler SP, Lindenbaum J: Etiology and diagnostic evaluation of macrocytosis. Am J Med Sci 2000;319:343–352.

45 Nexo E, Hvas AM, Bleie O, Refsum H, Fedosov SN, Vollset SE, Schneede J, Nordrehaug JE, Ueland PM, Nygard OK: Holo-transcobalamin is an early marker of changes in cobalamin homeostasis: A randomized placebo-controlled study. Clin Chem 2002;48:1768–1771.

46 Carmel R: Measuring and interpreting holo-transcobalamin (holo-transcobalamin II). Clin Chem 2002;48:407–409.

47 van Asselt DZ, Thomas CM, Segers MF, Blom HJ, Wevers RA, Hoefnagels WH: Cobalamin-binding proteins in normal and cobalamin-deficient older subjects. Ann Clin Biochem 2003;40:65–69.

48 Heaney ML, Golde DW: Myelodysplasia. N Engl J Med 1999;340:1649–1660.

49 Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C: Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982;51:189–199.

50 Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, Vallespi T, Hamblin T, Oscier D, Ohyashiki K: International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 1997;89:2079–2088.

51 Provan D, Weatherall D: Red cells. II. Acquired anaemias and polycythaemia. Lancet 2000;355:1260–1268.

52 Spivak JL: The blood in systemic disorders. Lancet 2000;355:1707–1712.

53 Kario K, Matsuo T, Kodama K, Nakao K, Asada R: Reduced erythropoietin secretion in senile anemia. Am J Hematol 1992;41:252–257.

54 Carmel R: Anemia and aging: An overview of clinical, diagnostic and biological issues. Blood Rev 2001;15:9–18.

55 Ershler WB: Biological interactions of aging and anemia: A focus on cytokines. J Am Geriatr Soc 2003;51(suppl):18–21.

56 Lipschitz DA, Udupa KB, Milton KY, Thompson CO: Effect of age on hematopoiesis in man. Blood 1984;63:502–509.

57 Nilsson-Ehle H, Swolin B, Westin J: Bone marrow progenitor cell growth and karyotype changes in healthy 88-year-old subjects. Eur J Haematol 1995;55:14–18.

58 Bagnara GP, Bonsi L, Strippoli P, Bonifazi F, Tonelli R, D'Addato S, Paganelli R, Scala E, Fagiolo U, Monti D, Cossarizza A, Bonafé M, Franceschi C: Hemopoiesis in healthy old people and centenarians: Well-maintained responsiveness of CD34+ cells to hemopoietic growth factors and remodeling of cytokine network. J Gerontol A Biol Sci Med Sci 2000;55:B61–B66.