



Universiteit
Leiden
The Netherlands

Anemia in old age

Elzen, W.P.J. den

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General discussion

Anemia is very common in older individuals. The prevalence of anemia rises with age to >20% in individuals aged 85 years and over.^{1,2} Increasingly, data become available that question the extrapolation of ‘common’ medical knowledge into the highest age groups. Since most studies on anemia have been performed in older persons aged 65 years and over and in selected patient groups only, the impact and etiology of anemia in very old individuals in the general population is largely unknown. Therefore, the aim of this thesis was to study the impact and etiology of anemia in the oldest old, in order to support the development of evidence-based diagnostic and treatment recommendations for anemia in old age. This chapter summarizes the main results presented in this thesis, discusses implications for clinical practice and includes recommendations for further research.

THE IMPACT OF ANEMIA

Risk marker or risk factor?

In our study population, anemia was associated with an increased mortality risk and functional decline (Chapter 2). Many other observational studies in older adults have also documented associations between anemia and a number of adverse outcomes.^{1,3-9} Although these associations have been recognized for years, it has been questioned whether anemia is an independent cause of disability and death (risk factor) or is merely a marker of underlying disease (risk marker).⁹⁻¹³

For clinical decision making, it is important to know whether these associations are truly causal. If they are, then it may be possible to decrease the risk of adverse outcomes in older individuals through prevention and correction of anemia. However, if these associations are not causal, increasing hemoglobin levels *per se* would not offer an opportunity for prevention of adverse outcomes such as frailty, disability, cognitive impairment, or early death in older anemic individuals, but could still be useful for prognostic purposes as it may identify those at high risk of a poor outcome.¹⁴

To determine whether anemia is causally related to these negative outcomes, we may use the 9 criteria presented by Sir Austin Bradford Hill.¹⁵ Observed associations may pass to a verdict of causation when the following criteria are fulfilled: (1) strength, (2) consistency, (3) specificity, (4) temporality, (5) biological gradient, (6) plausibility, (7) coherence, (8) experiment, and (9) analogy.¹⁵

In the past years, many studies have shown strong dose-dependent associations between anemia, functional decline and death,^{1,3-9} thereby fulfilling Bradford Hill’s criteria for strength, temporality and biological gradient. With regard to plausibility, it does not strike odd to suggest that anemia causally contributes to adverse outcomes by interfering with the oxygen delivery to the brain, heart and muscles.^{14,16,17}

Because chronic disease is a classic confounder in the association between anemia and functional decline or mortality, we applied rigorous statistical methods in Chapter 2 to adjust for the presence of known and unknown disease. Nonetheless, definite conclusions about the true effect of anemia on functional decline and mortality, even after adjustment for comorbidity, are hampered by two methodological issues: 1) there may be residual confounding by the presence of (subclinical) disease, resulting in an overestimation of the true effect of anemia on adverse outcomes, 2) anemia is an intermediary step in the causal pathway from chronic disease to functional decline and mortality.¹⁸ Therefore, difficulties with causality arise with criterium 2 on consistency and criterium 3 on specificity.

By adjusting for chronic disease we adjusted for a factor in the causal pathway that may have led to an underestimation of the true association between anemia and functional status and mortality. One way to solve these particular issues would have been to restrict the analyses to those without disease as this is the category of persons that was most likely not to have residual confounding by disease.¹⁹ Maraldi and colleagues performed a cross-sectional analysis of the baseline data of the Italian Group of Pharmacoepidemiology in the Elderly Study. The study consisted of 10,903 patients aged 65 years and over, admitted to the participating hospitals between 1993 and 1998. In analyses stratified by comorbidity severity, the association between anemia and disability was statistically significant only in patients with the lowest comorbidity levels.¹¹ Unfortunately, this restriction was not possible in our study population, since only a small number of participants was free of chronic disease.

These methodological issues call for the ultimate experiment (criterium number 8): a randomized controlled clinical trial. To find out whether anemia itself is causally related to mortality and functional decline, it would not suffice to select older patients with anemia and to detect and treat the underlying disease. If these patients show clinical improvement when compared to a control group, it would still be impossible to disentangle the independent effects of detecting and treating the underlying disease, and of correcting the anemia. Only a trial in which the role of chronic disease can be eliminated would help to answer whether anemia is an independent cause of anemia.^{14;20} In theory, one would want to investigate whether increasing hemoglobin to non-anemic levels in older people with anemia (e.g. with blood transfusions), regardless of the underlying disease, improves functional status and increases the length and quality of life. If so, anemia itself may indeed be considered a risk factor for adverse outcomes in older individuals. Only when such a trial has been performed, clear recommendations for clinical practice can be formulated. As it is not ethical to withhold patients with anemia from diagnosis and treatment of the underlying cause of the anemia, it will prove very difficult, if not impossible, to ever perform such a trial. Interestingly though, a strong argument in favor of the hypothesis that anemia itself is risk factor for functional decline and mortality is the fact that recombinant erythropoietin treatment has been shown to

be effective in raising hemoglobin levels and improving the quality of life in community-dwelling older persons with unexplained chronic anemia.²¹

A screening program for anemia in old age?

Since anemia is highly prevalent in old age and has potential clinical impact, one may argue that a screening program for anemia in old age is needed. In 1968, Wilson and Jungner formulated ten criteria for screening programs for disease.²² These criteria included items on the severity of the health problem, the availability of an accepted treatment, the availability of facilities for diagnosis and treatment, understanding of the natural history of disease, and agreement on treatment policy.²² Since anemia is very common in old age (27%, Chapter 2) and is associated with a number of negative outcomes, a screening program for anemia in the oldest old may have potential value, especially since a previous analysis in the Leiden 85-plus Study revealed that 76% of the participants with anemia were unknown to the general practitioner.²³ Prevention and correction of anemia may thus potentially decrease the risk of adverse outcomes in older individuals.

Since many of the Wilson and Jungner criteria are based on the presence of appropriate diagnosis and treatment, insight into the etiology of anemia in the oldest old is needed in order to develop evidence-based screening programs, and preventive and treatment strategies for anemia in old age. However, the benefit of and strategy applied for this screening program depend not only on whether a screening program for anemia fulfills all the Wilson and Jungner criteria including the availability of appropriate treatment but also on the impact of screening for anemia on society. As indicated previously, screening for anemia will reveal many older individuals with anemia, unknown to their treating physician. These older individuals will have to go through a number of (invasive) tests to find the cause of the anemia and may be confronted with aggressive and/or lifelong treatment strategies. Apart from studying the etiology of anemia in old age and cost-benefit of a screening program for anemia in old age, it is therefore also of vital importance to gain more insight into the GPs' and older persons' opinions with regard to screening for anemia.

THE ETIOLOGY OF ANEMIA

In medical text books such as Harrison's Principles of Internal Medicine,²⁴ iron deficiency, (undetected) blood loss, vitamin B12 deficiency, folate deficiency, renal failure, chronic disease, inflammation and myelodysplasia are stated as the classic causes of anemia in older people. We evaluated some of these classic causes of anemia in our study populations of 85-year-olds in Chapters 3 through 8.

Vitamin B12 deficiency

The consequences of vitamin B12 deficiency

In Chapter 3, we showed that folate deficiency and elevated homocysteine levels at age 85 years were associated with anemia at baseline and during follow-up, but vitamin B12 deficiency was not.

Most guidelines recommend measuring vitamin B12 levels in older patients with anemia.^{25;26} When low vitamin B12 levels are found (and folate is normal), hydroxocobalamin injections are given, often for many years. In this respect, it may seem surprising that low vitamin B12 levels do not associate with an increased risk of anemia in older individuals, especially since the biological role of vitamin B12 in hematopoiesis is well-defined.²⁷⁻³⁰ However, the results of our study are not the first to cast doubt on the relationship between low vitamin B12 levels and anemia in older individuals. We performed a systematic review to evaluate the association between subnormal vitamin B12 levels and anemia in older people in published literature to date (Chapter 4). Apart from 22 observational studies which showed inconsistent results with regard to the association between subnormal vitamin B12 levels or vitamin B12 deficiency and anemia in older subjects, we identified 3 well-designed placebo-controlled randomized trials that showed no effect of vitamin B12 supplementation on hemoglobin levels and MCV during follow-up in subjects with subnormal vitamin B12 levels at the start of the study. We concluded that the evidence of a positive association between subnormal vitamin B12 levels and anemia in older people is limited and inconclusive.

Interestingly, in an earlier report of the Leiden 85-plus Study it was shown that low vitamin B12 levels at age 85 years do not predict an accelerated deterioration in cognitive function.³¹ Similar conclusions were drawn in other observational studies and in several randomized controlled trials that showed no effect of vitamin B12 administration on cognitive function.^{32;33}

Clinical implications of these findings

The findings mentioned above do not at all imply that patients with pernicious anemia or food-vitamin B12 malabsorption should be withheld from vitamin B12 administration. Several non-placebo-controlled studies showed (large) increases in hemoglobin levels or hematocrit after vitamin B12 administration in patients with pernicious anemia or food-vitamin B12 malabsorption.^{34;35} However, apart from the undisputed reality of (pernicious) anemia, the clinical impact of a low vitamin B12 level in older people remains unclear, and accumulating evidence suggests that clinicians should at least reconsider the risks of a low vitamin B12 level before starting cyanocobalamin or hydroxocobalamin supplementation in the very old.³⁶ The total costs of vitamin B12 treatment in older individuals may be considered limited when compared to other types of medical treatment, but these low costs do not justify initiation and continuation of vitamin B12 treatment in older persons if not supported by medical evidence. In addition, it may distract attention from other

possible underlying causes. Additional proof of the (lack of) effectiveness of vitamin B12 treatment in older patients with anemia and subnormal vitamin B12 levels is a randomized double blind placebo-controlled trial in which older patients with anemia and subnormal vitamin B12 levels either receive usual care with hydroxocobalamin or placebo.

Folate deficiency and elevated homocysteine

Based on our findings in Chapter 3, early detection of folate deficiency by screening may identify older individuals at risk of developing anemia. The biochemical pathways suggest that folic acid supplementation is beneficial, but it is uncertain if folic acid fortification of grain and cereal products (as employed in the United States^{37;38}) has a positive effect on the incidence of anemia in the oldest old and should also be employed in the Netherlands.³⁹ This is a topic of interest for further studies.

Since serum levels of vitamin B12 and folate alone may not accurately reflect vitamin status at tissue level, we also studied the effect of elevated serum homocysteine concentration on anemia, which is considered to be a more sensitive marker of these deficiencies at the tissue level.⁴⁰⁻⁴² The strong association between elevated homocysteine levels and anemia disappeared after adjustment for the presence of folate deficiency. Elevated homocysteine levels will thus identify older subjects at increased risk of developing anemia, but do not seem to be causally related to anemia in old age. Since high homocysteine has also been shown to be a good predictor of cardiovascular mortality in old age,⁴³ homocysteine may have potential value in the identification of older individuals at increased morbidity and mortality risk. Not only have these findings to be validated in other cohorts, but the consequences for cardiovascular risk management have to be elucidated.⁴³

Iron deficiency and inflammation

In Chapter 5, we investigated the prognostic value of low ferritin levels in anemia in old age in the presence and absence of inflammation. Low ferritin was associated with lower hemoglobin levels and lower MCV, but this association was more pronounced in subjects with elevated C-reactive protein (CRP) levels than in subjects with normal CRP. These findings suggest that, despite ferritin's acute phase properties, it is very important to measure ferritin levels in older individuals, especially in those with infections or inflammation. It may be hypothesized that low ferritin is such a specific marker of iron status in individuals with inflammation *due to* its 'acute phase' properties: iron status must be poor when low ferritin levels are found in the presence of inflammation.

We presented two possible explanations for the occurrence of low ferritin levels in the presence of inflammation. First, in some patients with gastrointestinal tumors or other diseases, 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 may be another explanation for our findings. Pro-inflammatory cytokines, particularly interleukin 6, induce the production and secretion of hepcidin by hepatocytes. Hepcidin binds to the membrane protein ferroportin and induces its internalization and degradation in lysosomes, thereby blocking the export of iron from cells.⁴⁴ As serum hepcidin assays have recently been developed,⁴⁵ this hypothesis may be tested in the near future.⁴⁶ Depending on the outcomes of these additional studies, future diagnostic algorithms for anemia may incorporate markers of inflammation such as CRP or even hepcidin.⁴⁶ The results of these studies may also lead to innovative clinical trials, for instance by treating older patients with anemia of inflammation with anti-inflammatory agents or hepcidin antagonists.⁴⁷

Erythropoietin

Renal failure and anemia

Erythropoietin, produced by the kidney, is the main regulator of red blood cell production.⁴⁸ It is often thought that the high occurrence of anemia in very old people is caused by an age-related blunted erythropoietin response. In Chapter 6, we showed that erythropoietin levels in our study population of older individuals are comparable to erythropoietin levels found in studies with younger subjects.⁴⁹ However, in participants with severe renal failure (creatinine clearance below 30 ml/min), erythropoietin levels were indeed relatively low, indicating that older individuals in the general population with anemia and severe kidney dysfunction may be considered for erythropoietin substitution therapy, which has been shown to be effective in raising hemoglobin levels and improving the quality of life in (pre)dialysis and cancer patients.⁵⁰⁻⁵³

Mortality

In Chapter 7, we investigated the prognostic value of elevated erythropoietin on mortality in older individuals. We observed a dose-dependent positive association between increasing erythropoietin levels and mortality, independent of gender, creatinine clearance, hemoglobin level, comorbidity, smoking and C-reactive protein level. It is not exactly clear why elevated erythropoietin levels mark excess mortality. We proposed a number of potential physiological mechanisms that may underlie this association. Among others, elevated erythropoietin levels could be a physiological response to a chronically increased hypoxic stimulus due to yet undiagnosed subclinical disease.^{54;55} Elevated erythropoietin may also be compensating for removal of erythrocytes from the blood, either because of erythrocyte fragility, subclinical chronic hemolysis, or blood loss.^{54;55} Further studies are needed to shed light on the mechanisms involved and to identify the clinical and therapeutic implications of a high erythropoietin level in old age, especially since in the past years a number of unexpected nonhematopoietic functions of erythropoietin have been identified.⁵⁶

Unexplained anemia

In approximately one third of patients, the cause of the anemia is unknown; their anemia is ‘unexplained’.⁵⁷ Since older subjects with unexplained anemia often present with low leukocyte counts,^{58;59} myelodysplastic syndromes or other types of bone marrow failure may be the underlying diagnosis for unexplained anaemia.^{57;59-61}

Telomeres are DNA-protein complexes at the ends of chromosomes. Telomere length has been correlated with major age-related diseases such as dementia,⁶²⁻⁶⁵ myocardial infarction,⁶⁶ heart failure,⁶⁷ atherosclerosis,⁶⁸ and solid tissue tumours.⁶⁹ Earlier studies have also shown an association between shorter telomeres and myelodysplastic syndromes and other bone marrow failure syndromes.⁷⁰⁻⁷⁴ We therefore hypothesized that telomere length is a marker of hematopoietic aging and bone marrow failure, and as a result, would be associated with anemia in old age (Chapter 8). Both in the Newcastle 85-plus Study and in the first cohort of the Leiden 85-plus Study, we observed no differences in telomere length between participants with anemia and participants without anemia, nor did telomere length correlate with any other hematological parameter. Thus, in contrast to other age-related diseases, telomere length is not associated with anemia or any other hematological parameter in older individuals in the general population, despite the plausible biological mechanism underlying this association. To further investigate this intriguing matter, studies incorporating bone marrow biopsies are needed.

CONCLUDING REMARKS

Although researchers and clinicians have paid much attention to the clinical implications and pathophysiology of anemia in older individuals, the consequences and underlying pathophysiological mechanisms of anemia in the oldest old in the general population at large were largely unknown. Therefore, the aim of this thesis was to provide new insights into the impact and etiology of anemia in older individuals in the general population. In various chapters we succeeded in this aim, although many issues remain unsolved and many new questions have arisen.

An important finding presented in this thesis is that anemia in old age appears to be associated with an increased risk of death, independent of comorbidity, but the associated functional decline appears to be attributed mainly to comorbidity. Randomized controlled trials are needed to investigate whether anemia is a risk factor or risk marker of excess mortality and functional decline in older individuals (if proven ethical). In addition, while folate deficiency at age 85 years was associated with the development of anemia during follow-up, vitamin B12 deficiency was not. Nowadays, many older subjects with subnormal vitamin B12 concentrations receive hydroxocobalamin treatment. However, the results of our study indicate that trials are needed to verify whether older individuals with anemia and subnormal vitamin B12 levels should at all be treated with hydroxocobalamin.

Furthermore, we found that low ferritin was associated with the presence of anemia, but this association was more pronounced in participants with elevated CRP levels than in participants with normal CRP levels. Serum hepcidin measurements may shed light on this complicated interrelation between iron deficiency, inflammation and anemia. Additionally, we found that only in participants with severe renal failure, erythropoietin levels are relatively low and that elevated erythropoietin levels are associated with increased mortality, independent of hemoglobin. Further studies are needed to identify the clinical relevance and therapeutic implications of a low and a high erythropoietin level in older people in the general population. Besides, in contrast to other age-related diseases, telomere length appeared not to be associated with anemia in older individuals in the general population, despite the plausible biological mechanism underlying this association. Studies incorporating bone marrow biopsies are needed to investigate this further.

Finally, future studies should focus on improving the diagnostic algorithms for anemia in older individuals by looking into the additional diagnostic value of erythropoietin, homocysteine, methylmalonic acid, CRP or hepcidin in these algorithms. Since the prevalence of anemia is highest in the highest age groups, further studies are needed to elucidate the specific causes of anemia in these age groups. As current diagnostic and therapeutic guidelines are based on the classic notions of the etiology of anemia, the guidelines on anemia may have to be revisited for the highest age groups in the years to follow.

REFERENCES

1. Beghe C, Wilson A, Ershler WB. Prevalence and outcomes of anemia in geriatrics: a systematic review of the literature. *Am J Med.* 2004;116 Suppl 7A3S-10S.
2. Gaskell H, Derry S, Andrew MR, McQuay HJ. Prevalence of anaemia in older persons: systematic review. *BMC Geriatr.* 2008;81.
3. Izaks GJ, Westendorp RG, Knook DL. The definition of anemia in older persons. *JAMA.* 1999;281(18):1714-1717.
4. Chaves PH, Xue QL, Guralnik JM, Ferrucci L, Volpato S, Fried LP. What constitutes normal hemoglobin concentration in community-dwelling disabled older women? *J Am Geriatr Soc.* 2004;52(11):1811-1816.
5. Denny SD, Kuchibhatla MN, Cohen HJ. Impact of anemia on mortality, cognition, and function in community-dwelling elderly. *Am J Med.* 2006;119(4):327-334.
6. Zakai NA, Katz R, Hirsch C et al. A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the Cardiovascular Health Study. *Arch Intern Med.* 2005;165(19):2214-2220.
7. Culleton BF, Manns BJ, Zhang J, Tonelli M, Klarenbach S, Hemmelgarn BR. Impact of anemia on hospitalization and mortality in older adults. *Blood.* 2006;107(10):3841-3846.

8. Eisenstaedt R, Penninx BW, Woodman RC. Anemia in the elderly: current understanding and emerging concepts. *Blood Rev.* 2006;20(4):213-226.
9. Lipschitz D. Medical and functional consequences of anemia in the elderly. *J Am Geriatr Soc.* 2003;51(3 Suppl):S10-S13.
10. Patel KV. Epidemiology of anemia in older adults. *Semin Hematol.* 2008;45(4):210-217.
11. Maraldi C, Ble A, Zuliani G et al. Association between anemia and physical disability in older patients: role of comorbidity. *Aging Clin Exp Res.* 2006;18(6):485-492.
12. Zarychanski R, Houston DS. Anemia of chronic disease: a harmful disorder or an adaptive, beneficial response? *CMAJ.* 2008;179(4):333-337.
13. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med.* 2005;352(10):1011-1023.
14. Chaves PH. Functional outcomes of anemia in older adults. *Semin Hematol.* 2008;45(4):255-260.
15. Hill AB. The environment and disease: association or causation? *Proc R Soc Med.* 1965;58:295-300.
16. Chaves PH, Semba RD, Leng SX et al. Impact of anemia and cardiovascular disease on frailty status of community-dwelling older women: the Women's Health and Aging Studies I and II. *J Gerontol A Biol Sci Med Sci.* 2005;60(6):729-735.
17. Ferrucci L, Balducci L. Anemia of aging: the role of chronic inflammation and cancer. *Semin Hematol.* 2008;45(4):242-249.
18. Rothman KJ. Biases in study design. *Epidemiology An Introduction.* New York, Oxford University Press. 2002. 94-112.
19. Vandembroucke JP. When are observational studies as credible as randomised trials? *Lancet.* 2004;363(9422):1728-1731.
20. Paltiel O, Clarfield AM. Anemia in elderly people: Risk marker or risk factor? *CMAJ.* 2009.
21. Agnihotri P, Telfer M, Butt Z et al. Chronic anemia and fatigue in elderly patients: results of a randomized, double-blind, placebo-controlled, crossover exploratory study with epoetin alfa. *J Am Geriatr Soc.* 2007;55(10):1557-1565.
22. Wilson JMG, Jungner G. Principles and practice of screening for disease. World Health Organization, Public health papers ed. 1968.
23. de Craen AJ, Gussekloo J, Teng YK, Macfarlane PW, Westendorp RG. Prevalence of five common clinical abnormalities in very elderly people: population based cross sectional study. *BMJ.* 2003;327(7407):131-132.
24. Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL et al, editors. *Harrison's Principles of Internal Medicine*, 16 ed. New York, McGraw-Hill. 2004.
25. Kolnaar BGM, Van Wijk MAM, Pijnenborg L, Assendelft WJJ. Summary of the Dutch College of General Practitioners' practice guideline 'anaemia'. *Ned Tijdschr Geneeskd* 2003;147(40):1956-1961.

26. van Balen JAM, van Suijlen JDE, Rutten WPF et al. Landelijke Eerstelijns Samenwerkings Afspraak - Rationeel aanvragen van Laboratoriumdiagnostiek. Nederlands Huisartsen Genootschap, Nederlandse Vereniging voor Klinische Chemie, Samenwerkende Artsenlaboratoria Nederland. 2006.
27. Babior BM, Bunn HF. Megaloblastic Anemias. In: Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL et al, editors. *Harrison's Principles of Internal Medicine*, 16 ed. New York, McGraw-Hill. 2004. 602-607.
28. Martens JH, Barg H, Warren MJ, Jahn D. Microbial production of vitamin B12. *Appl Microbiol Biotechnol*. 2002;58(3):275-285.
29. Samson D, Halliday D, Chanarin I. Reversal of ineffective erythropoiesis in pernicious anaemia following vitamin B12 therapy. *Br J Haematol*. 1977;35(2):217-224.
30. Myhre E. Studies on megaloblasts in vitro. I. Proliferation and destruction of nucleated red cells in pernicious anemia before and during treatment with vitamin B 12. *Scand J Clin Lab Invest*. 1964;16307-319.
31. Mooijaart SP, Gussekloo J, Frolich M et al. Homocysteine, vitamin B-12, and folic acid and the risk of cognitive decline in old age: the Leiden 85-Plus Study. *Am J Clin Nutr*. 2005;82(4):866-871.
32. Ellinson M, Thomas J, Patterson A. A critical evaluation of the relationship between serum vitamin B, folate and total homocysteine with cognitive impairment in the elderly. *J Hum Nutr Diet*. 2004;17(4):371-383.
33. Malouf R, Areosa SA. Vitamin B12 for cognition. *Cochrane Database Syst Rev*. 2003;(3):CD004326.
34. Andres E, Kaltenbach G, Noel E et al. Efficacy of short-term oral cobalamin therapy for the treatment of cobalamin deficiencies related to food-cobalamin malabsorption: a study of 30 patients. *Clin Lab Haematol*. 2003;25(3):161-166.
35. Bolaman Z, Kadikoylu G, Yukselen V, Yavasoglu I, Barutca S, Senturk T. Oral versus intramuscular cobalamin treatment in megaloblastic anemia: a single-center, prospective, randomized, open-label study. *Clin Ther*. 2003;25(12):3124-3134.
36. den Elzen WP, Westendorp RG, Frolich M, de Ruijter W, Assendelft WJ, Gussekloo J. Role of Vitamin B12 in Anemia in Old Age – In reply. *Arch Intern Med*. 2009;169(12):168.
37. Food and Drug Administration. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. *Fed Regist*. 1996;618781-8797.
38. Tucker KL, Mahnken B, Wilson PW, Jacques P, Selhub J. Folic acid fortification of the food supply. Potential benefits and risks for the elderly population. *JAMA*. 1996;276(23):1879-1885.
39. Gezondheidsraad. Naar een optimaal gebruik van foliumzuur, publicatienr 2008/02 ed. Den Haag, 2008.

40. Stabler SP, Allen RH, Savage DG, Lindenbaum J. Clinical spectrum and diagnosis of cobalamin deficiency. *Blood*. 1990;76(5):871-881.
41. Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. *Am J Med*. 1994;96(3):239-246.
42. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency: a guide for the primary care physician. *Arch Intern Med*. 1999;159(12):1289-1298.
43. de Ruijter W, Westendorp RG, Assendelft WJ et al. Use of Framingham risk score and new biomarkers to predict cardiovascular mortality in older people: population based observational cohort study. *BMJ*. 2009;338a3083.
44. Nemeth E, Tuttle MS, Powelson J et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*. 2004;306(5704):2090-2093.
45. Kemna EH, Tjalsma H, Podust VN, Swinkels DW. Mass spectrometry-based hepcidin measurements in serum and urine: analytical aspects and clinical implications. *Clin Chem*. 2007;53(4):620-628.
46. Kemna EH, Tjalsma H, Willems HL, Swinkels DW. Hepcidin: from discovery to differential diagnosis. *Haematologica*. 2008;93(1):90-97.
47. Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. *Annu Rev Nutr*. 2006;26:323-342.
48. Adamson JW, Longo DL. Chapter 58. Anemia and Polycythemia: Introduction. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL et al, editors. *Harrison's Principles of Internal Medicine*, 17 ed. 2008.
49. Ble A, Fink JC, Woodman RC et al. Renal function, erythropoietin, and anemia of older persons: the InCHIANTI study. *Arch Intern Med*. 2005;165(19):2222-2227.
50. Cody J, Daly C, Campbell M et al. Recombinant human erythropoietin for chronic renal failure anaemia in pre-dialysis patients. *Cochrane Database Syst Rev*. 2005;(3):CD003266.
51. Evans RW, Rader B, Manninen DL. The quality of life of hemodialysis recipients treated with recombinant human erythropoietin. Cooperative Multicenter EPO Clinical Trial Group. *JAMA*. 1990;263(6):825-830.
52. Bohlius J, Wilson J, Seidenfeld J et al. Recombinant human erythropoietins and cancer patients: updated meta-analysis of 57 studies including 9353 patients. *J Natl Cancer Inst*. 2006;98(10):708-714.
53. Jones M, Schenkel B, Just J, Fallowfield L. Epoetin alfa improves quality of life in patients with cancer: results of metaanalysis. *Cancer*. 2004;101(8):1720-1732.
54. Ershler WB, Sheng S, McKelvey J et al. Serum erythropoietin and aging: a longitudinal analysis. *J Am Geriatr Soc*. 2005;53(8):1360-1365.
55. Price EA. Aging and erythropoiesis: current state of knowledge. *Blood Cells Mol Dis*. 2008;41(2):158-165.

56. Brines M, Cerami A. Erythropoietin-mediated tissue protection: reducing collateral damage from the primary injury response. *J Intern Med.* 2008;264(5):405-432.
57. Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood.* 2004;104(8):2263-2268.
58. Lipschitz DA, Mitchell CO, Thompson C. The anemia of senescence. *Am J Hematol.* 1981;11(1):47-54.
59. Lipschitz DA, Udupa KB, Milton KY, Thompson CO. Effect of age on hematopoiesis in man. *Blood.* 1984;63(3):502-509.
60. Rothstein G. Disordered hematopoiesis and myelodysplasia in the elderly. *J Am Geriatr Soc.* 2003;51(3 Suppl):S22-S26.
61. Pfeilstocker M, Karlic H, Nosslinger T et al. Myelodysplastic syndromes, aging, and age: correlations, common mechanisms, and clinical implications. *Leuk Lymphoma.* 2007;48(10):1900-1909.
62. Martin-Ruiz C, Dickinson HO, Keys B, Rowan E, Kenny RA, von Zglinicki T. Telomere length predicts poststroke mortality, dementia, and cognitive decline. *Ann Neurol.* 2006;60(2):174-180.
63. Panossian LA, Porter VR, Valenzuela HF et al. Telomere shortening in T cells correlates with Alzheimer's disease status. *Neurobiol Aging.* 2003;24(1):77-84.
64. von Zglinicki T, Serra V, Lorenz M et al. Short telomeres in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor? *Lab Invest.* 2000;80(11):1739-1747.
65. Yaffe K, Lindquist K, Kluse M et al. Telomere length and cognitive function in community-dwelling elders: Findings from the Health ABC Study. *Neurobiol Aging.* 2009;doi:10.1016/j.neurobiolagingn.2009.12.006.
66. Brouillette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol.* 2003;23(5):842-846.
67. Collerton J, Martin-Ruiz C, Kenny A et al. Telomere length is associated with left ventricular function in the oldest old: the Newcastle 85+ study. *Eur Heart J.* 2007;28(2):172-176.
68. Benetos A, Gardner JP, Zureik M et al. Short telomeres are associated with increased carotid atherosclerosis in hypertensive subjects. *Hypertension.* 2004;43(2):182-185.
69. Wu X, Amos CI, Zhu Y et al. Telomere dysfunction: a potential cancer predisposition factor. *J Natl Cancer Inst.* 2003;95(16):1211-1218.
70. Ball SE, Gibson FM, Rizzo S, Tooze JA, Marsh JC, Gordon-Smith EC. Progressive telomere shortening in aplastic anemia. *Blood.* 1998;91(10):3582-3592.
71. Boultonwood J, Fidler C, Kusec R et al. Telomere length in myelodysplastic syndromes. *Am J Hematol.* 1997;56(4):266-271.

72. Lange K, Holm L, Vang NK et al. Telomere shortening and chromosomal instability in myelodysplastic syndromes. *Genes Chromosomes Cancer*. 2010;49(3):260-269.
73. Ohyashiki JH, Iwama H, Yahata N et al. Telomere stability is frequently impaired in high-risk groups of patients with myelodysplastic syndromes. *Clin Cancer Res*. 1999;5(5):1155-1160.
74. Sashida G, Ohyashiki JH, Nakajima A et al. Telomere dynamics in myelodysplastic syndrome determined by telomere measurement of marrow metaphases. *Clin Cancer Res*. 2003;9(4):1489-1496.

