Anemia in old age

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Relation between erythropoietin, hemoglobin and renal function in the oldest old. The Leiden 85-plus Study

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ABSTRACT

Background In clinical practice, the occurrence of anemia in very old people is likely to be caused by an age-related low erythropoietin response. Erythropoietin response depends on renal function, which is often compromised in old age. The aim of our study was to unravel the relation between erythropoietin, hemoglobin and renal function in the oldest old.

Methods Within the Leiden 85-plus Study, an observational population-based prospective follow-up study, erythropoietin, hemoglobin and creatinine levels were determined at the age of 86 (n=491). Anemia was defined as Hb <12 g/dL for women and Hb <13 g/dL for men. Creatinine clearance was estimated with the Cockcroft-Gault formula.

Results The median (interquartile range) erythropoietin level was 10.2 mIU/mL (7.7-13.8 mIU/mL). In both anemic and non-anemic subjects the highest erythropoietin levels were found in subjects with the highest creatinine clearance. Subjects with creatinine clearance <30 mL/min had significantly lower hemoglobin levels (12 g/dL) than the subjects with creatinine clearance >60 mL/min (13 g/dL, $p<0.001$). Furthermore, participants with creatinine clearance <30 mL/min had significantly lower erythropoietin levels (9.1 mIU/mL) compared to subjects with creatinine clearance >60 mL/min (11.2 mIU/mL, $p_{trend}=0.05$).

Conclusion Despite compromised renal function in the oldest old, erythropoietin production in response to anemia is relatively intact. Only when creatinine clearance was lower than 30 mL/min, relatively low erythropoietin levels were found in relation with hemoglobin levels, suggesting a causal relationship.

INTRODUCTION

As often thought by clinicians, the occurrence of anemia in very old people is likely to be caused by an age-related blunted erythropoietin response. If low erythropoietin levels are causally related to the high prevalence of anemia in very old age, it needs to be elucidated whether the low erythropoietin levels are caused by the aging process itself or by the declining renal function.

Erythropoietin is synthesized mainly by the kidney, while approximately 10% has an extrarenal origin.[1] Erythropoietin is the main regulator of red blood cell production. The fundamental stimulus for erythropoietin production is impaired availability of oxygen for tissue metabolic needs. Impaired oxygen delivery to the kidney can result from a decreased red cell mass (anemia), hypoxemia due to diseases like chronic obstructive pulmonary disease, or rarely, impaired blood flow to the kidney due to renal artery stenosis. A hypoxemic state stimulates the oxygen sensor in peritubular interstitial cells near the proximal convoluted tubules, where erythropoietin is produced. Therefore, erythropoietin is inversely related to hypoxemia and hemoglobin levels.[2]

During chronic renal failure erythropoietin synthesis may be reduced when creatinine clearance falls below 60 mL/min in men and 40 mL/min in women.[3] After the age of 35, structural changes in the kidney associated with the aging process lead to an annual decrease in the glomular filtration rate of about 1 mL/min.[4-6] In contrast to this renal aging process, hematological senescence does not alter the red cell mass.[7] Therefore reference values for hemoglobin levels are not age-related.

The aim of our study was to unravel the relation between erythropoietin, hemoglobin and renal function in the oldest old. In the Leiden 85-plus Study, we assessed which older people suffered from a relative erythropoietin deficiency and whether it is necessary to establish age-related reference values of serum erythropoietin levels for this age category.

METHODS

Study population

The present study is embedded in the Leiden 85-plus Study, a population-based prospective follow-up study of persons aged 85 years and older. No selection criteria were applied. At baseline, all individuals were living in Leiden, the Netherlands. A total number of 599 subjects (response rate 87%) agreed to participate.[8] All participants were visited at their place of residence where interviews took place and venous blood samples were drawn. All measurements were obtained at age 86.

The Medical Ethical Committee of the Leiden University Medical Center approved the study and all participants provided informed consent for study participation.

Laboratory measurements

Blood samples were collected in sterile EDTA tubes. Measurements of hemoglobin were done in the LUMC with the fully automated system Sysmex XE-2100, TOA Medical Electronics, Kobe, Japan. For the present study, anemia was defined according to the criteria of the World Health Organization as hemoglobin levels lower than 12.0 g/dL for women and 13.0 g/dL for men.[9]

Serum erythropoietin levels were measured using an enzyme immunoassay (EIA), Immulite 2500, Siemens Medical Diagnostics, Tarrytown, NY, which has a sensitivity of 1.2 mU/mL and a coefficient of variation less then 6%. Creatinine was measured according to the Jaffe method using Hitachi 747, Tokyo, Japan. Creatinine clearance was estimated with the Cockcroft-Gault formula.[10] Although the Modification of Diet in Renal Disease equation (MDRD) is nowadays frequently used in clinical practice, up till now it has not been validated for the age above 70.[11] Furthermore, a recent Italian Study in 75-year-old subjects questions the use of the MDRD equation for this age category because it does not incorporate a strong effect of age as the Cockroft-Gault formula does.[12]

Ferritin, folate and vitamin B12 were determined in one batch using the Dual Count Solid Phase No-Boil Assay, Diagnostic Products Corp, Los Angeles, California.

Other characteristics

At baseline, a research nurse collected information concerning the demographic characteristics. The presence of comorbidity was defined as a history of cardiovascular diseases, diabetes, Parkinson's disease, chronic obstructive pulmonary disease, osteoarthritis, or malignancies.

Statistical analysis

Data are presented as number (percentages) for clinical characteristics and as median (interquartile range [IQR]) for continuous parameters. The differences in laboratory measurements between the sexes at baseline were determined by Mann-Whitney U tests for continuous variables and by Chi-Square tests for categorical variables. All subjects at age 86 were divided in three equal strata based on tertiles of erythropoietin representing a low, middle and high erythropoietin group. P-values for trend for differences within these groups were obtained by Jonckheere-Terpstra tests. Erythropoietin levels stratified for hemoglobin level and creatinine clearance at age 86 were visually depicted. For this purpose, all subjects were divided into 3 groups of creatinine clearance and 3 groups of hemoglobin based on clinically relevant criteria. The 3 groups of creatinine clearance were: low group <30 mL/min, middle group 30-60 mL/min and high group >60 mL/min. The 3

categories of hemoglobin levels were: low group <12 g/dL for women and <13.0 g/dL for men, middle group 12–14.5 g/dL for women and 13–15.5 g/dL for men and a high group >14.5 g/dL for women and >15.5g/dL for men.

The SPSS software (version 16.0.1, SPSS Inc, Chicago, Ill) was used for all statistical analyses. P-values lower than 0.05 were considered statistically significant.

RESULTS

Of the 599 participants enrolled in the study at age 85, 47 died before the age of 86 years, 39 refused further participation, 16 refused blood sampling and of 6 participants no erythropoietin measurement was available due to technical problems. Therefore, in these analyses, we included 491 participants aged 86 years.

In Table 1, baseline characteristics and laboratory measurements of the participants at age 86 are shown. The median (IQR) erythropoietin level was 10.2 mIU/mL (7.6-13.6 mIU/mL) for women and 10.2 mIU/mL (7.9-14.0 mIU/mL) for men (p=0.69), irrespective of hemoglobin level and renal function. The median (IQR) hemoglobin level was 12.8 g/dL (12.1-13.6 g/dL) for women and 13.4 g/dL (12.6-14.4 g/dL) for men (p<0.001). Renal function, as calculated by Cockroft-Gault formula, was lower in women (42 mL/min) than in men (46 mL/min) (p=0.002).

The prevalence of anemia was 23% (20% in women and 29% in men). The median (IQR) erythropoietin level in anemic subjects at age 86 (11.7 mIU/mL [9.0-16.4 mIU/mL]) was higher than the median erythropoietin level in non-anemic subjects (9.6 mIU/mL [7.5-12.8 mIU/mL], p<0.01). In both anemic and non-anemic subjects, the highest erythropoietin levels were found in subjects with the highest creatinine clearance, although statistical significance was not reached in anemic persons (p=0.06). Furthermore, in anemic subjects erythropoietin titers were significantly higher in those subjects with lower ferritin levels (p<0.01) (data not shown).

The association between hemoglobin and renal function over different erythropoietin levels is shown in Table 2. The highest erythropoietin levels were found in subjects with the lowest hemoglobin levels, and in those with the highest creatinine clearance (p_{trend} <0.001 and p_{trend}=0.03, respectively).

Table 1. Characteristics of subjects of the Leiden 85-plus Study at age 86

	Women (n=331)	Men (n=160)	P-value
General characteristics			
History of comorbidity*	231 (70)	111 (69)	0.904
Institutionalized	82 (25)	26 (16)	0.046
Previous and/or current smoking	97 (29)	135 (84)	<0.001
MMSE	26 (21-28)	27 (23-28)	0.035
Laboratory measurements			
Erythropoietin, mIU/mL	10.2 (7.6-13.6)	10.2 (7.9-14.0)	0.785
Lowest tertile	7.0 (6.2-7.7)	7.3 (6.0-7.9)	0.247
Middle tertile	10.3 (9.3-11.3)	10.0 (9.2-11.0)	0.260
Highest tertile	16.0 (13.6-18.8)	15.0 (13.4-18.0)	0.201
Hemoglobin, g/dL	12.8 (12.1-13.6)	13.4 (12.6-14.4)	<0.001
Low†	11.4 (10.6-11.7)	11.8 (11.2-12.3)	<0.001
Middle†	13.0 (12.6-13.6)	13.9 (13.4-14.6)	<0.001
High†	14.9 (14.7-15.4)	15.8 (15.7-16.3)	<0.001
Anemia‡	67 (20)	47 (29)	0.020
Erythrocytes, x 10^9/L	4.3 (4.0-4.6)	4.4 (4.1-4.7)	0.010
MCV, fL	91 (88-94)	92 (89-95)	0.011
Ferritin, µg/L	107 (58-177)	149 (79-236)	0.001
Folate, nmol/L	13 (10-16)	12 (10-15)	0.297
Vitamin B12, pmol/L	259 (210-344)	262 (194-343)	0.420
Creatinine, µmol/L	88 (76-100)	107 (95-127)	<0.001
Creatinine clearance§, mL/min	42 (36-50)	46 (39-53)	0.002
<30 mL/min	31 (8)	13 (8)	
30-60 mL/min	261 (82)	123 (80)	0.290
>60 mL/min	25 (10)	19 (12)	

Continuous parameters are presented as median (interquartile range). Categorial data are presented as numbers (percentages). P-values were obtained by Mann-Whitney U tests (continuous parameters) and Chi-square tests (categorical parameters).
Abbreviations: MMSE, Mini Mental State Examination; MCV, Mean Corpuscular Volume.
* Comorbidity was defined as a history of cardiovascular diseases, diabetes, Parkinson's disease, chronic obstructive pulmonary disease, osteoarthritis, or malignancies.
† 3 categories of hemoglobin levels; low <12.0 g/dL for women and <13.0 g/dL for men, middle 12.0 -14.5 g/dL for women and 13.0–15.5 g/dL for men, and high >14.5 g/dL for women and >15.5 g/dL for men.
‡ Anemia defined by the WHO criteria: for men Hb <13.0 g/dL, for women Hb <12.0 g/dL.
§ Creatinine clearance was obtained by the Cockcroft-Gault formula.

Table 2. Factors related with erythropoietin in all subjects at age 86

	Tertiles of Erythropoietin			
	Low N=164	Middle N=162	High N=165	Ptrend
Erythropoietin, mIU/ml	7.1 (6.1-7.8)	10.2 (9.3-11.2)	15.5 (13.6-18.3)	-
Anemia*	25 (15.2%)	36 (22.2%)	53 (32.1%)	<0.001
Hemoglobin, g/dL	13.3 (12.6-14.1)	13.1 (12.3-13.7)	12.8 (11.8-13.4)	<0.001
MCV, fL	91 (88-93)	92 (89-94)	91 (89-94)	0.34
Ferritin, mg/L	124 (75-235)	130 (78-215)	87 (41-170)	<0.001
Creatinine clearance†, mL/min	42.6 (35.8-49.9)	43.1 (36.9-52.6)	44.7 (37.7-51.4)	0.03

Continuous parameters are presented as median (interquartile range). Categorial data are presented as numbers (percentages). Ptrend was obtained by Jonckheere-Terpstra test.
Abbreviations: MCV; Mean Corpuscular Volume.
* Anemia defined by criteria of the World Health Organization.
† Creatinine clearance was obtained by Cockcroft-Gault formula.

When subjects were categorized using the clinical criteria generally used for describing chronic kidney dysfunction, median (IQR) erythropoietin level in non-anemic subjects was 10.5 mIU/mL (8.5-12.9 mIU/mL) for subjects with creatinine clearance >60 mL/min (n=35; 7%), and 9.8 mIU/mL (7.5-12.9 mIU/mL) when creatinine clearance 30-60 mL/min (n=308; 63%). For subjects with severe renal insufficiency (creatinine clearance <30 mL/min) median (IQR) erythropoietin level in non-anemic subjects was 8.3 mIU/mL (6.8-11.7 mIU/mL) (n=22; 4%).

The association between erythropoietin level, hemoglobin status and different creatinine clearance groups based on clinical criteria is shown in Figure 1. Participants with creatinine clearance <30 mL/min had significantly lower erythropoietin levels (9.1 mIU/mL) than subjects with creatinine clearance >60 mL/min (11.2 mIU/mL, p_{trend}=0.05). Furthermore, subjects with creatinine clearance <30 mL/min had significantly lower hemoglobin levels (12 g/dL) than subjects with creatinine clearance >60 mL/min (13 g/dL, p_{trend}<0.01).

DISCUSSION

In this large and representative population-based study of the oldest individuals, we found erythropoietin levels within the range as described in younger individuals. The physiological process of erythropoietin response to anemia in relation with relatively preserved renal function (creatinine clearance >30 mL/min) is still adequate in this age category. Only when renal function was severely reduced (<30 mL/min), a lower erythropoietin response in relation with the hemoglobin level was found.

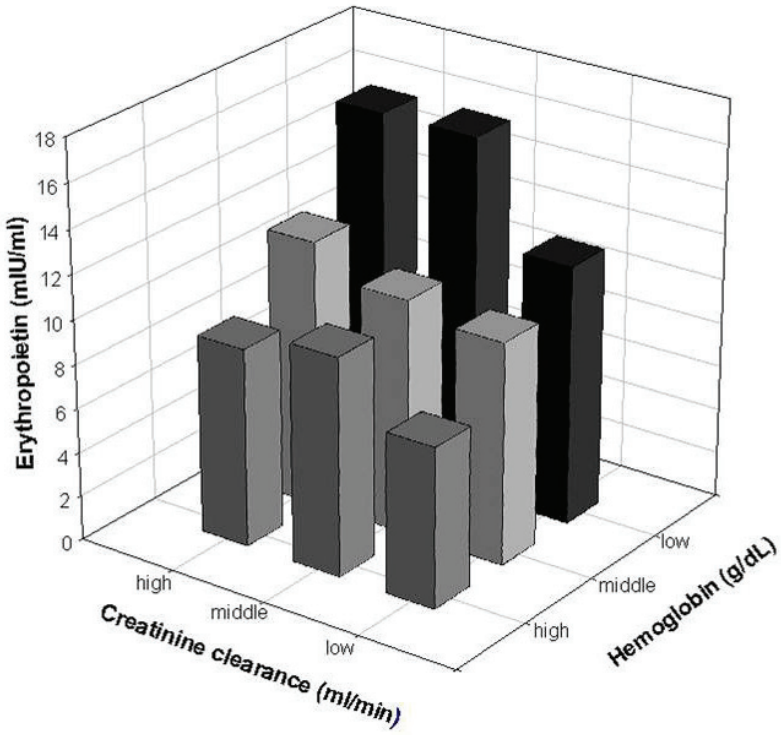

Figure 1. Erythropoietin levels according to hemoglobin level and creatinine clearance based on clinical criteria at age 86.
All subjects were divided into 3 groups of creatinine clearance; low group <30 ml/min, middle group 30-60 ml/min and high group >60 ml/min. All subjects were divided into 3 categories of hemoglobin levels: low group <12 g/dL for women and <13.0 g/dL for men, middle group 12 – 14.5 g/dL for women and 13 – 15.5 g/dL for men and a high group >14.5 g/dL for women and >15.5g/dL for men. The erythropoietin values on the vertical axis are median values.

Erythropoietin

The median erythropoietin level of the 86-year-old individuals was 10.2 mIU/mL. In the subjects with normal hemoglobin level and normal renal function (creatinine clearance >60 mL/min) the erythropoietin level was 10.5 mIU/mL. These erythropoietin levels are comparable with erythropoietin levels found in studies with younger subjects.[13;14] The 'InCHANTI Study' found almost similar erythropoietin levels in older persons with a mean age of 75 years.[15] These results indicate that, despite the aging process related alterations in the kidney mainly due to progressive loss of glomeruli and decline in renal blood flow,[16] there seems to be

no or little reduction in erythropoietin production in very old age. Therefore, it seems unlikely that for very old people new reference values for erythropoietin have to be established. Furthermore, we found no differences in erythropoietin level between men and women, confirming the results of previous studies.[13;15]

Anemia and erythropoietin

In this population of older individuals, the hemoglobin level at age 86 was 13 g/dL and the prevalence of anemia was 23%. Although anemia is a common condition in old age due to underlying disease, senescence itself does not seem to cause any reduction in hemoglobin level.[7] It has been estimated that individuals older than 85 years have a 2-3 fold greater prevalence of anemia compared with individuals aged 65 to 69 years.[17] In this respect, our study population can be considered as a representative population of the oldest old. We found that erythropoietin levels in anemic subjects at age 86 were significantly higher than erythropoietin levels in non-anemic subjects. Furthermore we showed an inverse relationship between hemoglobin and erythropoietin level, indicating that in the oldest old, the physiological response of erythropoietin production to anemia is not different from younger individuals. Furthermore, it indicates that the occurrence of anemia is not likely to be caused by an age-related blunted erythropoietin response, as often thought by clinicians, but due to other causes of anemia, requiring further clinical investigation. This is supported by our observation that ferritin levels in anemic subject were significantly lower compared to non-anemic subjects.

Renal function, anemia and erythropoietin

In our study population almost 80% of the population had moderate kidney dysfunction with a creatinine clearance of 30-60 mL/min as measured by the Cockroft-Gault formula. Renal function at age 86 was lower in women than in men. Both findings are in line with earlier reports.[5;16] Despite this moderate kidney dysfunction, hemoglobin levels (13.1 g/dL) in these participants were comparable with those with a relatively intact kidney function (creatinine clearance >60 mL/min, hemoglobin level 13.0 g/dL). The highest erythropoietin levels were found in those with the highest creatinine clearance (>60 mL/min), whether the subjects were anemic or not. As suggested in some reports,[15;18] the capacity to produce or secrete erythropoietin by the kidney is not altered by the aging process, but only by kidney function itself, irrespective of age. Relatively low erythropoietin values were only found when kidney function was severely reduced (creatinine clearance <30 mL/min).

Strength and limitations

These data are from a large, representative cohort of community dwelling very old people and therefore we were able to unravel the relation between erythropoietin level, renal function, and hemoglobin in the general population of oldest old. Although the estimation of creatinine clearance with the Cockroft-Gault formula is not the gold standard to measure renal function, it is a very widely used and

validated method for estimation of creatinine clearance and recently recommended to use in elderly populations instead of the MDRD.[4;10;12;19]

Conclusions

These results indicate that, despite reduced renal function due to aging related alterations in the kidney, there is no or little reduction in erythropoietin production in response to anemia in the oldest old. Only when creatinine clearance is lower than 30 mL/min a relatively low erythropoietin response is found. Therefore, very old subjects with anemia and a creatinine clearance of more than 30 mL/min should be considered for further clinical investigation. Anemia coinciding severe kidney dysfunction in this age group may be considered for erythropoietin substitution therapy instead.

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REFERENCES

1. Donnelly S. Why is erythropoietin made in the kidney? The kidney functions as a 'critmeter' to regulate the hematocrit. *Adv Exp Med Biol.* 2003;54373-87.
2. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med.* 2005;352(10):1011-1023.
3. Hsu CY, Bates DW, Kuperman GJ, Curhan GC. Relationship between hematocrit and renal function in men and women. *Kidney Int.* 2001;59(2):725-731.
4. Fehrman-Ekholm I, Skeppholm L. Renal function in the elderly (>70 years old) measured by means of iohexol clearance, serum creatinine, serum urea and estimated clearance. *Scand J Urol Nephrol.* 2004;38(1):73-77.
5. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc.* 1985;33(4):278-285.
6. van Bemmel T, Woittiez K, Blauw GJ et al. Prospective study of the effect of blood pressure on renal function in old age: the Leiden 85-Plus Study. *J Am Soc Nephrol.* 2006;17(9):2561-2566.
7. Izaks GJ, Westendorp RG, Knook DL. The definition of anemia in older persons. *JAMA.* 1999;281(18):1714-1717.
8. der Wiel AB, van Exel E, de Craen AJ et al. A high response is not essential to prevent selection bias: results from the Leiden 85-plus study. *J Clin Epidemiol.* 2002;55(11):1119-1125.
9. Nutritional anaemias. Report of a WHO scientific group. *World Health Organ Tech Rep Ser.* 1968;4055-37.
10. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* 1976;16(1):31-41.

11. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med.* 1999;130(6):461-470.
12. Pizzarelli F, Lauretani F, Bandinelli S et al. Predictivity of survival according to different equations for estimating renal function in community-dwelling elderly subjects. *Nephrol Dial Transplant.* 2009;24(4):1197-1205.
13. Kario K, Matsuo T, Nakao K. Serum erythropoietin levels in the elderly. *Gerontology.* 1991;37(6):345-348.
14. Powers JS, Krantz SB, Collins JC et al. Erythropoietin response to anemia as a function of age. *J Am Geriatr Soc.* 1991;39(1):30-32.
15. 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.
16. Epstein M. Aging and the kidney. *J Am Soc Nephrol.* 1996;7(8):1106-1122.
17. 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.
18. Musso CG, Musso CA, Joseph H et al. Plasma erythropoietin levels in the oldest old. *Int Urol Nephrol.* 2004;36(2):259-262.
19. Friedman JR, Norman DC, Yoshikawa TT. Correlation of estimated renal function parameters versus 24-hour creatinine clearance in ambulatory elderly. *J Am Geriatr Soc.* 1989;37(2):145-149.