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Introduction

General introduction

Our society's population is ageing rapidly. Nowadays, more than 15% of the Dutch population, 2.5 million persons, is aged 65 years and over and this is expected to increase to 25% in 2040. (1) Unfortunately, not all of these older subjects spent their life in good health. To strive for high quality cure and care for these "complex" older people, knowledge of and research in these aged persons is essential. On the one hand, increasingly data become available that the physiological processes in the oldest old subjects may be distinct from those in young and middle-aged individuals. Therefore, to extrapolate research findings, validated in large studies done with selected younger individuals, into the older populations can be misleading. On the other hand, some organs are les affected than others by the ageing process. For example renal function decreases linearly with increasing age, however there is limited effect of increasing age on bone marrow capacity.

This thesis focuses on the physiological aspects of the interesting triad renal function, erythropoietin production and haemoglobin levels at old age. The decrease in renal function is one of the most relevant functional defects that occurs with increasing age. Erythropoietin (EPO) is produced within the kidney. Furthermore, EPO is the hormone that regulates red blood cell production. Red blood cells develop in the bone marrow and circulate for about 100–120 days in the body. Haemoglobin is the iron-containing oxygen-transport protein within the red blood cells and with increasing age haemoglobin seems to remain stable. Therefore lower haemoglobin levels are not "normal" at old age with increased morbidity and mortality as a result. To develop treatment strategies in the very old, research into the physiology of ageing is needed in population based studies of older subjects. In this thesis we focus on physiological aspects as well as on consequences of decreased renal function and the impact of changes in EPO and haemoglobin levels at old age.

Renal function at old age

A decrease in renal function is one of the most important functional defects that occurs with increasing age. Kidney function is stable until age 30 to 40 and then declines linearly at an average rate of about 8ml/min/1.73m^2 per decade. (2) Although in clinical practice, the glomerular filtration rate (GFR) is considered to be the best overall reflection of renal function, it cannot be measured easily in daily clinical practice. Therefore GFR is usually estimated from equations (eGFR) such as the classic Cockroft-Gault formula (C-G) (3) and the Modification of Diet and Renal Disease equation (MDRD). (4) More recently

the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) was suggested as a more accurate estimate of GFR, especially for the relative high ranges of eGFR. (5-7) However, none of these measures are well validated in large samples of older populations. Moreover, impaired renal function has important clinical implications since it has been associated with increased cardiovascular complications and all cause and cardiovascular mortality. (8,9)

Erythropoietin

Erythropoietin (EPO) is primarily produced in the kidney and is the principle regulator of red blood cell production. (10) Decreased oxygen availability in the kidney, due to for example anemia or renal hypoperfusion, triggers the production of erythropoietin within the kidney. Conflicting data are available concerning EPO levels at old age. On the one sight it has been suggested that the availability of the kidney to secrete EPO declines with ageing and as a consequence anemia in older subjects is attributed to the decreased levels of erythropoietin. (11) On the other hand, the limited available studies suggest that erythropoietin levels of older subjects are comparable with those of younger individuals. (12,13) Whether endogenous erythropoietin levels are inadequate or nearly normal in elderly is important in clinical practice, because of therapeutic decision making about administrating exogenous erythropoietin for the correction of anemia.

Haemoglobin

Anemia, defined as low(er) haemoglobin levels (14), is a common clinical condition in the elderly (15,16) and with advancing age associated with unfavourable events like functional dependency, cardiovascular events, cognitive decline and impaired survival. (17-20) Anemia at old age is not simply a result of diminished hematopoiesis, since there is only little effect of ageing on bone marrow function. (21) Iron deficiency and chronic renal failure are the most common causes of anemia in older subjects. Other well known causes in clinical practice for anemia are folate and vitamin B12 deficiency. Furthermore, several epidemiological studies have found that up to 30% of anemia cases in the older population is unexplained, even when extensive clinical information is available. (22-24) Nowadays, this type of anemia is classified as "unexplained anemia". The underlying pathophysiological mechanisms of unexplained anemia have yet to be established and it's clinical relevance for older subjects needs to be determined.

Aim and outline of this thesis

In this thesis physiological aspects of the triad haemoglobin, renal function and erythropoietin at very old age are described. Furthermore, the prognostic consequences of changes in haemoglobin, renal function and erythropoietin in a cohort of oldest old subjects is assessed.

Chapter 2 presents the results of calculating renal function in old age by using three different formulae: Cockroft-Gault, MDRD and CKD-EPI equation and to find the best estimate of GFR (eGFR) at old age. The haematopoietic capacity at old age was studied in chapter 3 in two independent, population-based cohorts as well as the relationship between haemoglobin and mortality in oldest old subjects was investigated. The effect of increasing age on renal function and serum levels of haemoglobin and erythropoietin (EPO) in randomly selected individuals in the range of 30-100 years was described in chapter 4. Since high erythropoietin levels have been shown to predict the risk of death among patients with chronic heart failure, in chapter 5 the prognostic value of elevated erythropoietin levels on mortality among very elderly people in the general population was assessed. In chapter 6 the independent predictive value of clinical markers of inflammation in relation to mortality, both vascular and nonvascular, was studied in very old participants, since there predictive value have already been shown in middle-aged populations. Given that in approximately 30% of older persons with anemia, the cause of the anemia is unexplained, in chapter 7 we assessed the clinical differences between older subjects with explained and unexplained anemia and investigated whether these subjects have different mortality patterns compared to subjects without anemia.
Finally, in chapter 8 is assessed whether higher haemoglobin levels predict length of hospital stay after hip fracture surgery in elderly subjects. The goal of treating anaemia in older patients who have undergone hip fracture surgery is to enhance functional recovery. In chapter 9 the findings and the possible clinical implications of all presented studies are summarized with brief recommendations for clinicians working with older patients.

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Performance of Cockroft-Gault, MDRD and CKD-EPI in estimating prevalence of renal function and predicting survival in the oldest old

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Abstract

Introduction The question for prevalence estimation and validation of the various eGFRs in old age is still under debate. To assess renal function with increasing age, we estimated mean eGFR, in subjects aged 20-85 years. Furthermore, we assessed prevalence of eGFR in a population-based sample of 85 year olds and investigated the performance of these eGFRs in predicting mortality in the oldest old.

Methods Renal function with increasing age was assessed in subjects aged 20-85 years from the Bronovo Study Cohort. We estimated prevalences of eGFRs and mortality risks in a population-based study of persons aged 85 years and older, the Leiden 85-plus Study. The GFRs were estimated by three different formulas.

Results After the age of 70 years, the C-G tended to give relatively lower eGFRs. An eGFR < 60 was found in 90% of the subjects aged 85 years as calculated by C-G, in 55% of the subjects using MDRD and in 68% of the 85 year old subjects as calculated by CKD-EPI. When renal function was <30 ml/min/1.73m^2, an increased mortality risk was observed by C-G (HR 1.9 (95% CI 1.1-3.3)), by MDRD (HR 3.5 (95% CI 1.8-6.7)), whereas by CKD-EPI significance was not reached (HR 2.4 (95% CI 0.9-6.4)).

Conclusion Our study demonstrates that in subjects above age 70, C-G gives lower estimates of renal function when compared to MDRD and CKD-EPI. Furthermore, prevalence of renal dysfunction (CKD stage 1-3) at age 85 years was highest for C-G (90%), lowest for MDRD (55%), and 68% for CKD-EPI. Moreover, we found that in subjects aged 85 years MDRD predicted mortality best.

Introduction

Chronic kidney disease (CKD) coinciding with impaired renal function is predominantly a disease of the elderly and is associated with an increased risk for all cause and cardiovascular mortality, even after controlling for known risk factors. (1,2) Furthermore, renal impairment also affects safety of many common drugs used in older people. The glomular filtration rate (GFR) is considered to be the best overall reflection of renal function, but is not easily measured in daily practice. Therefore creatinine clearance is usually assessed by the classic Cockroft-Gault formula (C-G) and an estimate of glomerular filtration rate is often calculated by the Modification of Diet and Renal Disease equation (MDRD). (3,4) More recently the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) has been suggested as a more accurate estimate of eGFR (5), especially in the relative high ranges of eGFR (CKD stage 1 and 2). (6,7) However, these measures have not been used to assess the impact of eGFR on truly estimating renal function in older populations, and they are not well validated in the elderly. Since it is difficult to measure GFR in a large population based group of old people, the question of prevalence estimation and validation of the various eGFRs in this age group is still unanswered.

Compared to the MDRD and CKD-EPI estimates of GFR, an important characteristic of the C-G formula is the inclusion of total body weight in the equation, as a reflection of muscle mass, the main determinant of creatinine generation. (3) With increasing age, body composition changes with decreasing muscle mass and increasing fat tissue as characteristic features, resulting in decreased lean body mass in very old age. (8,9) These age related changes might have important effects on creatinine clearance as calculated by the C-G formula in older individuals. In contrast to the classic C-G formula, the MDRD and CKD-EPI equations incorporates body surface area, resulting in eGFRs per 1,73 m^2 body surface area.

Since it is virtually impossible to validate the various eGFRs in large population based samples of oldest old people by measuring GFR, for example by creatinine or inulin clearance, it is of great importance to determine the best measure of GFR in older people. First, to assess renal function with increasing age, we estimated mean creatinine clearance by C-G, and eGFR by MDRD and CKD-EPI equations, in 10 year age groups of subjects aged 20-85 years. Then, we estimated prevalence of eGFR in a population-based sample of 85 year olds and investigated the performance of these eGFRs in predicting mortality in the oldest old.

Methods

Study population

To investigate renal function as calculated by the C-G, MDRD and CKD-EPI equations, a cohort of subjects aged 20 to 85 years was used (study 1). This cohort was originally established to set reference values for laboratory measurements for various age categories in the Bronovo Hospital, The Hague, The Netherlands (10,11), a general hospital affiliated with Leiden University Medical Center, Leiden, the Netherlands. There were no inclusion criteria. Exclusion criteria were pregnancy, diabetes mellitus, use of oral contraceptives, vitamin- or iron supplements, and oral anti-coagulants. From all participants a venous blood sample was drawn. The Medical Ethical Committee of Bronovo Hospital accorded the study and all participants provided informed consent to study participation.

For studying prevalence of renal dysfunction based on the three formulas and their performance in predicting all cause mortality, the population of the Leiden 85-plus Study was used (study 2). The Leiden 85-plus Study is a population-based prospective follow-up study of persons aged 85 years and older. There were no selection criteria other than age. At baseline, all individuals were living in Leiden, The Netherlands. A total number of 599 subjects (response rate 87%) agreed to participate. (12) All participants were visited at their place of residence where interviews took place, height and weight measurements were done and venous blood samples were drawn. After inclusion all subjects were followed for mortality until February 2009.The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the study and all participants provided informed consent for study participation.

Laboratory measurements

All blood samples were collected in sterile EDTA tubes. Plasma creatinine concentrations were determined in the Bronovo Hospital (study 1) with Synchron LX-20, Beckman Coulter and in the LUMC (study 2) according to the Jaffe method using Hitachi 747, Tokyo, Japan.

Creatinine clearance and Glomular Filtration Rate equations

Three equations for renal function were used in our analysis (Table 1): the Cockcroft-Gault formula (C-G) (3), the four variable Modification of Diet in Renal Disease equation (MDRD) (4), and the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI). (5)

Table 1. Formulas of C-G, MDRD and CKD-EPI

	Gender	Serum creatinine*	eGFR (ml/min/1.73m^2)
Cockroft-Gault	Female	All	((140- age) x bodyweight / serum creatinine) x 0.85
	Male	All	(140- age) x bodyweight / serum creatinine
MDRD	Female	All	175 x (serum creatinine/88.4)$^{-1.154}$ x age$^{-0.203}$ x 0.742
	Male	All	175 x (serum creatinine/88.4)$^{-1.154}$ x age$^{-0.203}$
CKD-EPI	Female	≤ 62	144 x (serum creatinine/88.4/0.7)$^{-0.329}$ x (0.993)^{age}
	Female	> 62	144 x (serum creatinine/88.4/0.7)$^{-1.209}$ x (0.993)^{age}
	Male	≤ 80	141 x (serum creatinine/88.4/0.7)$^{-0.411}$ x (0.993)^{age}
	Male	> 80	141 x (serum creatinine/88.4/0.7)$^{-1.209}$ x (0.993)^{age}

*Serum creatinine is stated in µmol/L.
Abbreviations; eGFR: estimated glomerular filtration rate; MDRD: modification of diet in renal disease formula; CKD-EPI: chronic kidney disease epidemiology equation.
Because all our individuals were of the Caucasian race the multiplier factor for race was not applied.

Chronic Kidney Disease

CKD was defined according to the K/DOQI staging. (13) CKD stage 3 has been sub-divided into 30-44 (stage 3a) and 45-59 ml/min/1.73m^2 (stage 3b) as there is evidence of graded increase in mortality risk (Table 2). (14,15)

Table 2. Stages of chronic kidney disease

GFR	CKD stage	Description
≥ 60	Stage 1 and 2	Kidney damage with normal or mildly decreased GFR
45-59	Stage 3a	Moderately decreased GFR
30-44	Stage 3b	Moderately decreased GFR
< 30	Stage 4 and 5	Severely decreased GFR or kidney failure

Adapted from K/DOQI clinical practice guidelines (13)
GFR is stated in ml/min/1.73m^2
CKD stage 3 has been sub-divided into 30-44 (*stage 3b*) and 45-59 ml/min/1.73m^2 (*stage 3a*) as there is evidence of graded increase in mortality risk (9,14)

Mortality

Mortality data of the Leiden 85-plus Study (study 2), recorded between the start of the study, 1 September 1997, and 1 February 2009, were obtained from the municipal registry, which are publicly available. For the deceased participants the cause of death was obtained from Statistics Netherlands. We obtained permission to collect this data and only the primary cause of death on the death certificate was used in our analyses.

Statistical analyses

Data are presented as number (percentages) for clinical characteristics and as median (interquartile range) for continuous parameters. The association between measures of renal function and mortality was analyzed with sex-adjusted Cox proportional hazard models. Differences in laboratory measurements between the different categories of eGFR as well as between sexes were determined by Mann-Whitney tests. SPSS software (version 16.0.1, SPSS Inc, Chicago, Ill) was used for statistical analyses. P-values lower than 0.05 were considered statistically significant.

Results

Creatinine clearance and eGFR in subjects aged 20-85 years

Characteristics of subjects in study 1 are reported in Table 3. The study sample comprised 242 subjects, 125 (52%) women and 117 (48%) men.

Table 3. Characteristics of the Bronovo study cohort (study 1)

	Age category (years)					
	21-30 (n=42)	31-40 (n=43)	41-50 (n=43)	51-60 (n=52)	61-70 (n=41)	71-85 (n=21)
Female, n (%)	23 (55)	22 (51)	23 (54)	25 (50)	23 (56)	9 (43)
Creatinine (µmol/L)	76 (71-87)	85 (74-91)	82 (72-97)	86 (74-99)	83 (74-93)	87 (76-97)
Cockroft-Gault (ml/min)	117 (97-127)	112 (95-138)	97 (82-112)	87 (77-96)	78 (68-85)	57 (52-82)
MDRD (ml/min/1.73m^2)	94 (85-102)	86 (77-92)	78 (72-87)	75 (66-84)	72 (66-82)	69 (58-77)
CKD-EPI (ml/min/1.73m^2)	104 (93-111)	93 (83-99)	82 (75-92)	78 (68-87)	71 (65-83)	65 (54-73)

Data presented are as median (IQR), unless otherwise stated.
Abbreviations: MDRD; Modification of Diet in Renal Disease equation, CKD-EPI; Chronic Kidney Disease Epidemiology Equation.

In subjects aged 21 to 30 years, mean creatinine clearance as calculated by Cockroft-Gault formula was 117 ml/min, mean eGFR by MDRD formula was 94 ml/min/1.73m^2 and by CKD-EPI equation was 104 ml/min/1.73m^2. In the oldest age category, 71- 85 years, creatinine clearance calculated by Cockroft-Gault formula was 57 ml/min, eGFR by MDRD formula 69 ml/min/1.73m^2 and by CKD-EPI equation 65 ml/min/1.73m^2. With increasing age, a significant decline of renal function was observed in C-G, MDRD and CKD-EPI (all $p<0.01$). Figure 1 shows estimates of mean renal function as assessed with C-G, MDRD and CKD-EPI in the age categories. The three lines of the C-G, MDRD, and CKD-EPI cross at age 70 years. Before the age of 70 years, renal function as assessed by C-G is above the MDRD and CKD-EPI, indicating that before age 70 years eGFR assessed with the C-G formula tends to give relatively higher eGFRs.

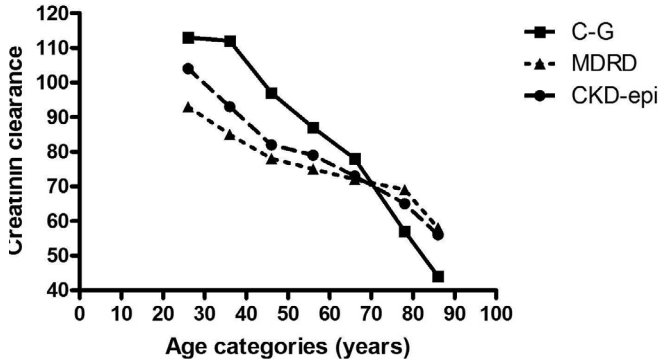

Figure 1. Renal function in subjects of different age categories. Modification of Diet in Renal Disease (MDRD) clearance and Chronic Kidney Disease Epidemiology Collaboration clearance (CKD-EPI) are expressed in ml/min/1.73m^2 and Cockroft-Gault (C-G) clearance in ml/min.

After the age of 70 years, the line of the renal function as assessed with the C-G formula is below the MDRD and CKD-EPI, indicating that after age 70 years, eGFRs assessed with the C-G formula tend to give relatively lower eGFRs.

Prevalence of renal dysfunction in the oldest old

Characteristics of subjects in study 2 are reported in Table 4. Sixty-seven percent of the subjects was female and 30% had no comorbid illness. Median weight of all subjects was 69.5 kg and the median body mass index (BMI) was 26.7 kg/m^2. Median creatinine clearance, calculated by C-G, was 43 ml/min. Using this formula, in 496/550 subjects (90%) a creatinine clearance lower than 60 ml/min was found. Furthermore, of these 85 years old subjects, the median eGFR, as calculated by MDRD, was 58 ml/min/1.73m^2 and

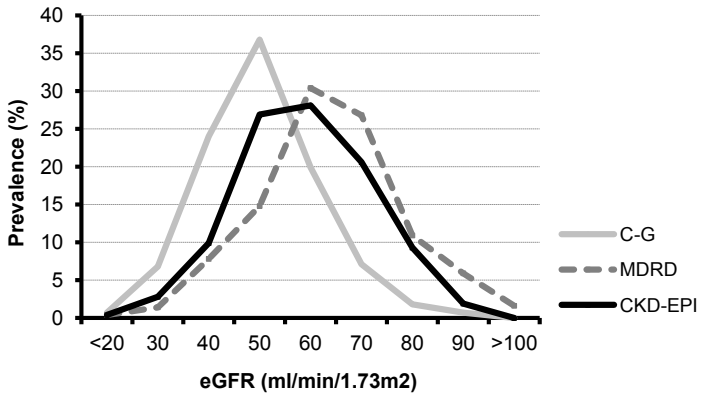

Figure 2. Proportion of subjects in the various categories of eGFRs, calculated by C-G, MDRD and CKD-EPI. Abbreviations: C-G; Cockroft-Gault formula, MDRD; Modification of Diet in Renal Disease, CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration clearance.

Table 4. Characteristics of subjects aged 85 of the Leiden 85-plus Study

	n=562
Female	377 (67)
Comorbid illness	398 (70)
Institutionalized	104 (18)
MMSE	26 (22-28)
Weight (kg)	69.5 (61.5-78.3)
Length (cm)	159 (154-166)
Body Mass Index (weigth/m^2)	26.7 (24.2-29.9)
Body Surface Area (m^2)*	1.72 (1.61-1.85)
Serum Creatinine (µmol/L)	92 (81-108)
Cockroft-Gault (ml/min)∫	43 (37-51)
>60 ml/min	54 (10)
30-60 ml/min	454 (83)
<30 ml/min	42 (7)
MDRD (ml/min/1.73m^2)	58 (49-68)
>60 ml/min/1.73m^2	254 (45)
30-60 ml/min/1.73m^2	298 (53)
<30 ml/min/1.73m^2	10 (2)
CKD-EPI (ml/min/1.73m^2)	53 (46-62)
>60 ml/min/1.73m^2	179 (32)
30-60 ml/min/1.73m^2	365 (65)
<30 ml/min/1.73m^2	18 (3)

Continuous parameters are presented as median (IQR).
Categorial data are presented as number (%)
*Body Surface Area = calculated by DuBois's formula
∫ in 12 subjects weight was not available to calculate Cockroft-Gault clearance
Abbreviations: MMSE; Mini Mental State Examination, MDRD; Modification of Diet in Renal Disease, CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration clearance.

308/562 subjects (55%) were having an eGFR lower than 60 ml/min/1.73m^2. Moreover, when calculating GFR by CKD-EPI formula, a median eGFR of 53 ml/min/1.73m^2 was found and in 383/562 subjects (68%) eGFR was under 60 ml/min/1.73m^2.

We determined the proportion of subjects in the various categories of renal function, calculated by C-G, MDRD and CKD-EPI. Figure 2 illustrates that the C-G estimates result in higher prevalences of renal failure compared to MDRD and CKD-EPI.

Reclassification of subjects in the various eGFR categories assessed by MDRD and CKD-EPI is shown in Table 5. When the MDRD formula was used, 308/562 (54.8%) subjects were labelled as CKD stage 3 or worse (eGFR< 60 ml/min/1.73m^2). When the CKD-EPI

Table 5. Number of subjects in categories of CKD assessed by MDRD and CKD-EPI

CKD-EPI	MDRD					
	<15	15-29	30-59	60-89	>90	
<15	**2**	0	0	0	0	2
15-29	0	**8**	*8*	0	0	16
30-59	0	0	**290**	*75*	0	365
60-89	0	0	0	**170**	*9*	179
>90	0	0	0	0	**0**	0
Total	2	8	298	245	9	562

Numbers in **bold** indicate those subjects who do not change CKD category on the basis of eGFR assessed with MDRD and CKD-EPI. *Italic* figures indicate the numbers of subjects who change into a different CKD stage.
MDRD and CKD-EPI clearance are stated in ml/min/1.73m^2.
Abbreviation: CKD; Chronic Kidney Disease, MDRD; Modification of Diet in Renal Disease, CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration clearance.

formula was used, 383/562 (68.1%) subjects were classified as CKD stage 3 or worse. Overall, 92 subjects moved up one CKD stage when MDRD was used in stead of CKD-EPI. There were no subjects that moved up when CKD-EPI was used in stead of MDRD. The largest reclassification was for the subjects within the CKD-EPI category 30-59 ml/min/1.73 m^2. Of the 365 subjects within this category, 75 were reclassified into the MDRD category 60-89 ml/min/1.73 m^2.

The associations between the different eGFRs and mortality risk are shown in Table 6. For the calculations of the mortality risk, eGFRs above 60 ml/min/1.73 m^2 were set as reference group. With the C-G formula, an almost twofold increased risk for all-cause mortality was found for subjects with a renal function lower than 30 ml/min/1.73m^2 (HR 1.9 (95% CI 1.1-3.3)). An increased mortality risk was also observed by calculating renal function by MDRD, when renal function was under 30 ml/min/1.73m^2 (HR 3.5 (95% CI 1.8-6.7), and when renal function was between 30-44 ml/min/1.73m^2 (HR 1.6 (95% CI 1.2-2.2). When renal function was estimated with CKD-EPI, subjects with renal function

Table 6. Relative mortality risks in categories of eGFR for C-G, MDRD and CKD-EPI

	Hazard ratio (95% CI)			
	< 30 ml/min	30-44 ml/min	45-59 ml/min	>60 ml/min
C-G	1.9 (1.1-3.3)*	1.3 (0.9-1.9)	1.0 (0.7-1.5)	1
MDRD	3.5 (1.8-6.7)**	1.6 (1.2-2.2)**	1.1 (0.9-1.3)	1
CKD-EPI	2.4 (0.9-6.4)	1.3 (0.9-1.8)	1.1 (0.9-1.5)	1

All hazard ratios are adjusted for sex.
C-G clearance is in ml/min, MDRD and CKD-EPI clearance is in ml/min/1.73m^2.
Abbreviations: C-G; Cockcroft-Gault, MDRD; Modification of Diet in Renal Disease, CKD-EPI; Chronic Kidney Disease Epidemiology Collaboration clearance.
*=P<0.05, ** P≤ 0.01 compared to the reference group (>60 ml/min).

lower than 30 ml/min/1.73m^2 had a similar increased all-cause mortality risk compared to MDRD, but significance was just not reached, HR 2.4 (95% CI 0.9-6.4).

Discussion

The results of our study are threefold. First, our study demonstrates that in subjects under age 70 years, C-G gives higher estimates of renal function when compared to MDRD and CKD-EPI, while in subjects above age 70, C-G gives lower estimates of renal function when compared to MDRD and CKD-EPI. Second, prevalence of renal dysfunction (CKD stage 3-5) at age 85 years was highest for C-G (90%), lowest for MDRD (55%), and 68% for CKD-EPI. Third, we found that in subjects aged 85 years MDRD predicted mortality best. These results suggest that at very old age the MDRD formula might be the best estimate for eGFR, since the MDRD formula is most discriminative in predicting mortality.

eGFR in various age categories

We showed that C-G, MDRD and CKD-EPI formula provide different estimates of renal function in various age categories. Under the age of 70, C-G clearance relatively overestimated renal function compared to eGFRs calculated by both MDRD clearance and CKD-EPI, whereas above the age of 70 creatinine clearance assessed with C-G formula resulted in relatively lower values. Our results are in line with earlier studies with older individuals (all mean age <85 years) (16,17), and also comparable with another community based study with younger participants (mean age of 75 years). (18) The difference in mean age of the participants is the most plausible explanation between these study findings.

The discrepancy in renal function calculated by the C-G formula and the MDRD and CKD-EPI equations may be explained by the intrinsic design of the estimates. In comparison with the MDRD and CKD-EPI equations, in the C-G formula the body weight is included next to the creatinine and age. In old age, lean body mass is reduced secondary to both sarcopenia and to increasing fat tissue. (8,9) The MDRD and CKD-EPI equations are adjusted for body surface area (BSA) (19), resulting in an eGFR value per 1.73 m^2 BSA. Therefore, ageing may also have an effect on this adjusted eGFR since, next to the changes in body weight, both women and men loose height with increasing age, resulting in a decline in BSA in old age. (20) In the Leiden 85-plus Study mean BSA was 1.72 m2, suggesting that the BSA adjustment used in the MDRD and CKD-EPI estimates may, however, be appropriate in this very old population. It may be questioned whether it is appropriate to index the C-G formula for BSA, because weight is already included in the equation as a variable. Therefore, the use of the C-G formula in clinical practice for very old individuals is questionable.

eGFR in the oldest old

Recently, the CKD-EPI, has been introduced in clinical practice, because of the possible inadequacies of the C-G and MDRD equations. (4,5) Our study shows that implementation of the CKD-EPI formula has consequences for very old subjects. Above the age of 70, eGFR calculated by CKD-EPI formula is underestimating renal function in comparison with eGFR calculated by MDRD, although not as much as the C-G formula. Compared with the classic C-G equation, introduction of the CKD-EPI formula will lower the amount of older individuals with CKD. Whereas based on these results, implementation of CKD-EPI formula would raise the number of older individuals with CKD on the basis of eGFR estimated with the MDRD formula, with as a consequence more hospitalizations, costs and also other therapeutic implications.

A recently published large population based study of over a half million UK people of all ages (21) found that introduction of the CKD-EPI formula would reduce the prevalence of CKD in subjects < 70 years, but would raise the prevalence of CKD in the over 70 year old group. Furthermore, another report with particular emphasis of eGFR and the effect of age found that among the very elderly CKD-EPI may actually increase CKD prevalence estimates. (22)

Although there are several studies with younger individuals that suggest that the CKD-EPI equation more accurately categorizes individuals (5,7) and although the US National Kidney Foundation has already recommended the adoption of the CKD-EPI formula for routine eGFR reporting by laboratories in the USA (23), based on our results and others (21,22,24-26), more research in the older individuals is warranted, before the CKD-EPI can be implemented in clinical practice for the oldest old age categories, in order to prevent unnecessary diagnostic procedures, therapeutic interventions and medical costs.

Mortality risks and eGFR at old age

Since one goal of estimating renal function in clinical practice is to obtain estimates of deaths risk in various stages of CKD, it seems logical to use the equation that provides the best prediction of these outcome, especially in older individuals. (1,2) Therefore, we examined the association between the three different assessment methods for renal function and mortality in old age and found the MDRD equation to be best predictive for mortality. Subjects with MDRD < 45 ml/min/1.73m^2 had higher mortality risks compared to renal function calculatd by C-G or CKD-EPI formulae. Moreover, in subjects with creatinine clearance < 30 ml/min/1.73m^2 calculated by MDRD formula, a 3.5 increased risk of mortality was found.

Our findings are in contrast with a large Italian study of 942 community dwelling subjects. (18) The participants of this study, the InCHIANTI study, had a mean age of 75 years. They found that only the C-G and not the MDRD equation was predictive for mortality.

Since estimating equations C-G and MDRD both incorporate age in the formula, a plausible explanation for the discrepancy between findings of the two studies is the difference in mean age of the participants. In our study we only included oldest old subjects, all aged 85 years. However, a study with hospitalized older individuals in the Netherlands (mean age 78 year) showed results similar to ours. (27) Furthermore, in line with our findings, a large British cohort study of people aged 75 years and older, showed a increased mortality risk with MDRD < 45 ml/min/1.73m^2. (15) Data of very old community-dwelling very subjects (≥ 85 years) and the prediction of all cause mortality by C-G and especially MDRD and CKD-EPI formulae are scarce.

Strong points and limitations

This is one of the few studies evaluating the effect of three different estimation methods for renal function in very old individuals in a population-based setting with a very high participation rate (87%) and with complete follow-up. This permits us to generalize our conclusions to very old people in the general population at large. Unfortunately, in both study cohorts, we did not have 24-hour urine collections for the measurement of creatinine clearance, although accuracy of urine collection at home done in a very old study population can be discussed. Moreover, accurate GFR measurements using inulin or iothalamate infusions are undoable for large scale study populations.

Conclusion

In conclusion, estimation of renal function in very old persons can be facilitated by GFR equations, although C-G, MDRD and CKD-EPI all have their own limitations. We found that after age 70 years, C-G gives lower eGFRs and might therefore overestimating the number of individuals having CKD in comparison with both MDRD and CKD-EPI after the age of 70 years. Moreover, our results suggest that the MDRD formula might be the best estimate for eGFR in the oldest old followed by the CKD-EPI formula, since the MDRD formula is the best in predicting mortality. Our study suggests that implementation of CKD-EPI formula would raise the number of older individuals with CKD in comparison with the MDRD formula, with consequences for therapeutic decision making procedures and resulting in more referrals to nephrologists. Therefore, more research in older individuals is urgently needed, before the CKD-EPI can be implemented in clinical practice for the oldest old age categories.

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3

Haematopoietic capacity and exceptional survival
The Leiden Longevity Study

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Abstract

Introduction Anemia has been associated independently of underlying diseases, with increased mortality in older persons. Furthermore, it has previously been demonstrated that nonagenarian siblings from the Leiden Longevity Study are genetically enriched for longevity. We questioned whether genetic enrichment for survival is dependent on sustained normal hematopoietic capacity in old age.

Methods Prospective follow-up study of two independent, population-based cohorts; the Leiden Longevity Study and the Leiden 85-plus Study. From the Leiden Longevity Study 1001 nonagenarians with familial longevity were included. As age-matched controls 260 nonagenarians without longevity were used from the Leiden 85-plus Study. Hematological measurements like haemoglobin, leucocytes, and thrombocytes were performed for all subjects with and without familial longevity. Standardised mortality ratios, linear regression and left censored Cox regression were used for statistical analysis.

Results Mortality in nonagenarians with familial longevity was 28 % lower than in nonagenarians from the general population (SMR 0.72, 95%CI 0.65-0.79, $p<0.001$). No differences were found between haemoglobin, leucocyte and thrombocyte count in nonagenarians with and without familial longevity (all $p>0.3$). Nonagenarians with familial longevity had greater mortality risk when anemia was present (sex adjusted hazard ratio 1.78, 95% CI 1.47-2.16, $p<0.001$). No relationship was found between leucocytes, thrombocytes, and mortality in either study group (all $p>0.2$).

Conclusion Haematopoietic capacity cannot explain the significant better survival of nonagenarians with familial longevity, but in those with familial longevity, anemia may contribute to mortality.

Introduction

The incidence of anemia rises sharply with age, with reported prevalences of up to 40% in octagenerians. (1) There are two possible explanations for anemia in elderly people. First, anemia can be a result of diminished hematopoiesis, although there is limited effect of increasing age on bone marrow capacity. (2) Second, anemia can be a reflection of concurrent disease, but in several population-based studies, anemia has been associated independently of underlying diseases, with increased mortality in older persons. (3) Studies of relationships of leucocytes and thrombocytes with lifespan have shown inconsistent results. (4)

Previous studies have consistently indicated that genetic factors can explain approximately 25% of the variation in lifespan. (5-7) It has previously been demonstrated that nonagenarian siblings from the Leiden Longevity Study are genetically enriched for longevity. (8) Twin studies have shown that haematological parameters have a strong genetic component, indicating that genetic factors explain 37%, 62%, and 57% of the variance in haemoglobin, leucocytes, and thrombocytes. (9,10) The question is whether genetic enrichment for survival is dependent on sustained normal hematopoietic capacity in old age.

In the Leiden Longevity Study, there was a unique opportunity to assess whether haematological parameters in nonagenarians are associated with higher mortality risks and whether nonagenarians with familial longevity have higher serum levels of haemoglobin, leucocytes and thrombocytes than age-matched population controls.

Methods

Study populations

All study subjects with familial longevity came from long-living families in the Netherlands. A representative example of a pedigree of a family of the Leiden Longevity Study is shown in Figure 1. A family could participate if at least two siblings were long-living. There were four inclusion criteria for the elderly subjects with familial longevity: men had to be 89 years or older and women had to be aged 91 and older, there had to be at least one living brother or sister who fulfilled the first criterion and was willing to participate, the siblings had to have the same biological mother and father, and the parents of the sibling pairs had to be Dutch and Caucasian. In total over 450 sibships and their offspring and spouses were included and venous blood samples were drawn. All were recruited between 2002 and 2005 and were followed up for mortality until February 2007.

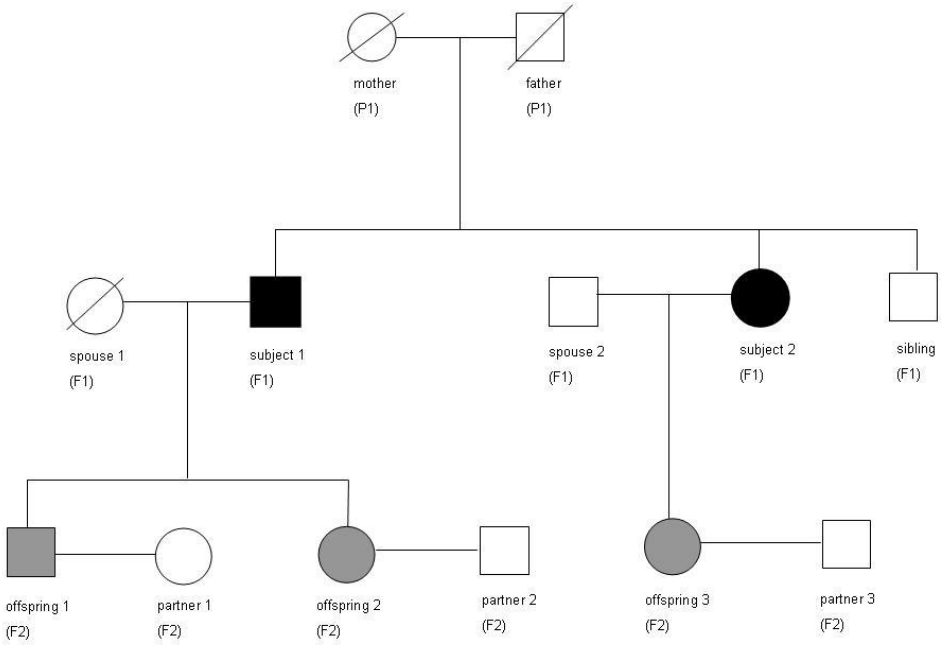

Figure 1. Representative pedigree of the Leiden Longevity Study. The black symbols show the ascertained long-living subjects and the grey symbols represent their offspring. P1, F1 and F2 represent the three generations included in the study.

A population control group of similar age for the long-living familial nonagenarians was constructed from the nonagenarians of the Leiden 85-plus Study. The Leiden 85-plus Study is a prospective, population based follow-up study of persons aged 85 years and older without familial longevity. At baseline, all individuals were living in Leiden, The Netherlands; 599 subjects participated. All participants were visited at their place of residence where interviews took place and a venous blood sample was drawn. All subjects used in our analysis were followed from age 90 onwards for mortality.

Because the probability of reaching the age of 90 in the Netherlands is 0.5%, hypothetically three subjects would overlap in both study cohorts, but in practice only two subjects were overlapping. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved both studies, and all participants provided informed consent for study participation.

Laboratory measurements

Blood samples were collected in sterile ethylenediaminetetraaceteric acid tubes. Measurements of haemoglobin, leucocytes, and thrombocytes for the subjects with familial longevity were performed in the Leiden Medical Diagnostical Center (SCAL) with the

automated system Siemens ADVIA 1200 system (SMSD, Tarrytown, NY). For the subjects without familial longevity the haematological measurements were done in the Leiden University Medical Center with the fully automated Sysmex XE-2100 system (TOA Medical Electronics, Kobe, Japan). Quality assurance indicated that there were no differences between the laboratories in the levels of haemoglobin and the leucocytes, but, thrombocyte counts were 3% higher in the SCAL and 2% lower in the LUMC than in the general Dutch population. Therefore, thrombocyte values were standardized to the Dutch population mean before the analysis.

Anemia was defined according to the criteria of the World Health Organization. (11) The normal reference interval for haemoglobin concentration defined at 7.5 to 10.0 mmol/L (120-160 g/L) for women and at 8.1 to 11.2 mmol/L (130-180 g/L) for men. Leucopenia was defined as absolute number of leucocytes less than 4.0 x 10^9/L and thrombopenia was defined as absolute number of thrombocytes less than 150 x 10^9/L.

Statistical analysis

The statistical analysis proceeded in various stages. First, the mortality of the nonagenarians with familial longevity was compared with that of the general Dutch population using standardized mortality ratios (SMRs). The SMR is the ratio of the observed number of deaths in the study relative to the expected number of deaths calculated from the mortality rates from the general population, adjusted for sex, age distribution, and calendar period. Second, the mortality of the subjects with familial longevity was compared with that of the subjects without familial longevity using left- censored Cox regression adjusted for sex. Third, sex-specific cross-sectional analysis of differences in haemoglobin, leucocyte, and thrombocyte counts of long-living elderly people and elderly people without familial longevity of the Leiden 85-plus Study was done with linear regression adjusted for age. Fourth, all subjects were classified as anemic, leucopenic, or thrombopenic according to the criteria of the World Health Organisation. The association between anemia, leucopenia, and thrombopenia in nonagenarians with and without familial longevity was assessed with left-censored Cox regression and was visually depicted using a Kaplan-Meier survival curve. Standardised mortality ratios and left-censored Cox regression were calculated using STATA, version LP 9.0 (Stata Corp, College Station, TX). All other analyses were performed with SPSS for Windows (version 12.0.1, SPSS Inc., Chicago,Ill).

Results

The study sample of nonagenarians with familial longevity of the Leiden Longevity Study comprised 622 women and 379 men. The age matched population controls with-

out familial longevity of the Leiden 85-plus Study comprised 188 women and 72 men (Table 1).

Table 1. Characteristics of nonagenarians with and without familial longevity stratified according to sex.

	Women		p-value	Men		p-value
	Familial longevity (n=622)*	No familial longevity (n=188)¶		Familial longevity (n=379)*	No familial longevity (n=72)¶	
Age, mean (SE)	93.3 (0.1)	90 (0)	<0.001	91.8 (0.1)	90 (0)	<0.001
Institutionalized, n (%)	NA	16 (9)	-	NA	9 (13)	-
Follow-up, years, mean (SE)	2.7 (1.3)	2.1 (0.9)	-	2.5 (1.3)	2.5 (1.7)	-
Haemoglobin, mmol/L, mean (SE)	8.1 (0.1)	8.2 (0.1)	0.08	8.4 (0.1)	8.1 (0.1)	0.10
Leucocytes, $x10^9$/L, mean (SE)	7.1 (0.1)	6.8 (0.2)	0.20	7.8 (0.4)	7.1 (0.9)	0.51
Thrombocytes, $x10^9$/L, mean (SE)	241 (2.6)	244 (4.6)	0.53	220 (3.8)	218 (7.1)	0.76

* Subjects recruited from the Leiden Longevity Study
¶ Subjects recruited from the Leiden 85-plus Study
Abbreviations: SE: standard error; NA: not available.

Mortality risk of the nonagenarians with familial longevity of the Leiden Longevity Study and of the general Dutch population in 2005 is visually depicted in Figure 2. The standardised mortality ratio for all 1001 nonagenarians with familial longevity compared with that of the general population was 0.72 (95% confidence interval (CI) = 0.65- 0.79), indicating 28% lower mortality in the study sample than the general Dutch population. For the familial long-living women from the Leiden Longevity Study, the standardised mortality ratio was 0.70 (95% CI = 0.62- 0.79). For the familial long-living elderly men the standardised mortality ratio was 0.74 (95% CI= 0.63-0.87).

The overall relative mortality risk of the nonagenarians with familial longevity compared with that of the control nonagenarians of the Leiden 85-plus Study was 0.61 (95% CI = 0.49-0.75). The mortality risk did not change after excluding subjects who were institutionalized 0.63 (95% CI = 0.48-0.90).

Hematological characteristics of nonagenarians with and without familial longevity are shown in Table 2. No differences were found between the mean hemoglobin, leucocyte, and thrombocyte concentrations of the female and male nonagenarians with and without familial longevity. For all subjects, the difference in hemoglobin, leucocytes and thrombocytes was not significant (all $p > 0.5$).

Subjects with familial longevity had an increased risk for all-cause mortality when they were anemic (sex-adjusted relative risk 1.71, 95% CI = 1.47-2.07, $p<0.001$). The greater risk

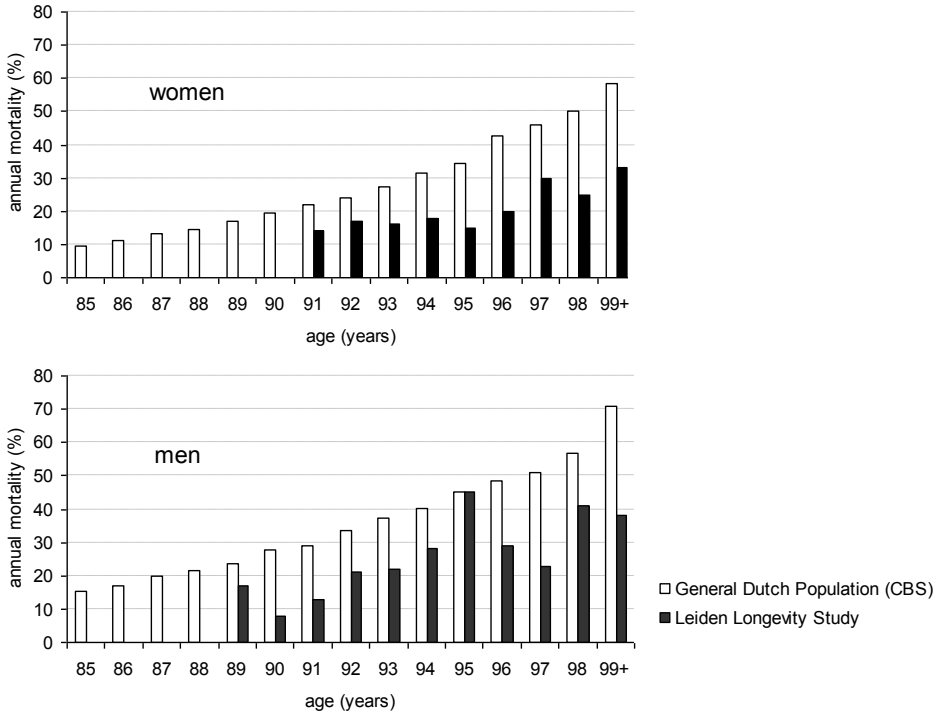

Figure 2. Mortality rates in elderly with familial longevity of the Leiden Longevity Study and the general Dutch population in women and men.

for mortality was even more pronounced in the non-familial nonagenarians with anemia (sex-adjusted relative risk 2.16, 95% CI = 1.54-3.02, p<0.001) (Figure 3). The p-value for the difference in hazard ratios between studies was 0.24. When haemoglobinwas analyzed as a continuous variable in the analysis, a hazard ratio of 0.74 (95% CI = 0.68-0.82, p<0.001) was found per unit increase in haemoglobin in the familial longevity subjects. In the non-familial subjects, the hazard ratio was 0.71 (95% CI = 0.60-0.84, p<0.001).

Table 2. Relationship between anemia, leucopenia, and thrombocytopenia and mortality in nonagenarians with and without familial longevity.

	Familial longevity*		No familial longevity¶	
Haematological parameters	Hazard Ratio (95% Confidence Interval)	*P*-value		
Anemia	1.71 (1.41-2.07)	<0.001	2.16 (1.54-3.02)	<0.001
Leucopenia	1.49 (0.88-2.52)	0.14	1.12 (0.49-2.53)	0.79
Thrombopenia	1.39 (0.97-1.99)	0.07	0.82 (0.44-1.53)	0.55

* Subjects recruited from the Leiden Longevity Study

¶ Subjects recruited from the Leiden 85-plus Study

Hazard ratios are age and sex adjusted.

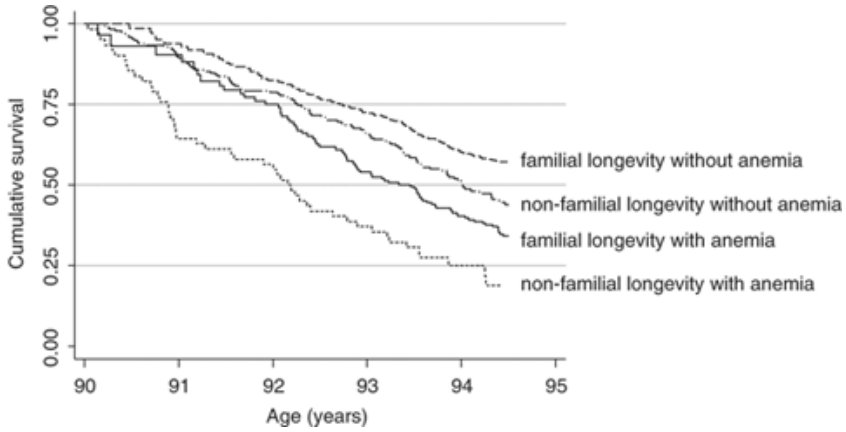

Figure 3. Graphical representation of the relationship between anemia and mortality from age 90 onwards in subjects with and without familial longevity.

Leucopenia and thrombopenia were present in a low percentage in both sexes of the long-living nonagenarians (< 7 %), and there was no association of leucopenia and thrombopenia with mortality risk (all $p > 0.1$) (Table 2).

Discussion

These results indicate that nonagenarian men and women with familial longevity have better survival compared to age-matched elderly people from the general population. No differences were found in haemoglobin level, leucocyte, and thrombocyte count between subjects with and without familial longevity. It was also found that familial long-living elderly men and women with anemia had higher mortality than subjects with normal levels. For leucopenia and thrombopenia, no greater mortality risk was found.

The better survival of elderly people with familial longevity of the Leiden Longevity Study than of the elderly subjects from the general Dutch population was in line with expectations. Earlier analysis of family members of this exceptional cohort of long-lived individuals already suggested a clear survival advantage. (8) When following the members of this cohort, a significantly lower mortality risk was found than in age-matched controls from the Leiden 85-plus study.

No significant differences were foung in serum haemoglobin levels in members of families with familial longevity compared to population controls in both men or women. If

higher haemoglobin levels had caused the better survival of elderly people with familial longevity, a difference in mean haemoglobin level between the two populations in both generations would have been expected. Therefore, hemoglobin levels are unlikely to explain the greater survival of subjects with familial longevity. It is likely that factors other than haemoglobin level genetically enriched the participants of the Leiden Longevity Study for better survival.

Elderly subjects with familial longevity had almost twice the mortality risk when they were anemic than non-anemic subjects in both sexes. This association between anemia and mortality has been studied before. These studies have also shown an association between anemia and greater mortality in elderly men and women without familial longevity. (3,12-14) Although subjects with and without familial longevity had similar levels of haematological parameters, the effect of these parameters can be differential. As shown in Table 2, the risks for mortality due to anemia were greater in both study groups, but the hazard ratio for mortality was more pronounced in subjects without familial longevity. Because of these greater mortality risks in all anemic elderly subjects, haemoglobin levels below normal are a reason for further clinical investigation in medical practice and may not be considered simply a result of "normal aging".

No significant differences in leucocyte and thrombocyte counts were found in the families with familial longevity and their population controls in men or women. Moreover, no greater mortality risk was found in subjects of familial longevity with leucopenia or thrombopenia. These data are at odds with the limited reported literature on mortality risk and white blood cell count or platelet count. One study showed that leucocyte count was an independent risk factor for all-cause mortality. (15) The study subjects were adults with a mean age of 56.7 years and of Asian origin. No recent literature was found about blood platelet count and mortality in elderly subjects. One article reported an association with low platelet count and mortality in middle-aged men with a mean age of 50. (16) This study is therefore not comparable with that of the current study.

Although the prevalence of anemia was 24% in the elderly women and approximately 40% in the elderly men with familial longevity, the prevalence of thrombopenia and leucopenia was far less in either of the sexes (2-7%). Previous studies have also shown little effect of aging on human bone marrow function. (2,17) Therefore, it is likelythat long-living subjects, have the same bone marrow reserve capacity as population controls. The reported heritability estimates for haemoglobin, leucocytes, and thrombocytes of respectively 0.37, 0.62, and 0.57, respectively, also indicate that haemoglobin levels are more volatile and that anemia therefore reflects underlying morbidity more than leucopenia and thrombopenia do.

Strength and limitations

The strength of the current study was that it compared two cohorts of the oldest-old: one group of nonagenarians with familial longevity and one cohort resembling the general elderly population, all in relative large numbers.

A possible limitation of this study is that the possibility of selection bias cannot be excluded. Conditions associated with poor outcome, such as diseases at baseline, living conditions, and cognition, could not be compared in either study population, although the mortality risk did not change when institutionalized elderly subjects were excluded.

Conclusion

In conclusion, nonagenarian men and women with familial longevity have better survival than age-matched elderly from the general population. No differences were found in haemoglobin level, leucocyte and thrombocyte count between elderly with and without familial longevity. It was found that all long-living elderly people anemia had higher mortality than subjects with normal level; therefore haemoglobin levels below normal are a reason for further clinical investigation in medical practice. No greater mortality risk was found for leucopenia and thrombocytopenis. Therefore, haematopoietic capacity cannot explain the significant better survival of nonagenarians with familial longevity, and lower haemoglobin levels may reflect concurrent disease.

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4

Stable haemoglobin and increasing erythropoietin levels despite age related renal dysfunction

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submitted

Abstract

Introduction Lower haemoglobin levels in old age are believed to be caused by a decline in production of erythropoietin (EPO) by age-related renal dysfunction. On the other hand, there is some evidence that EPO levels remain stable with advancing age or even increase. We investigated the association between increasing age and serum levels of haemoglobin, EPO and renal function in healthy individuals in different age categories.

Methods The Bronovo Study cohort consists of 268 healthy individuals in the range of 30-100 years, living in The Hague, the Netherlands. In this observational study, haemoglobin, reticulocytes, erythrocytes and EPO measurements were performed for all subjects. Jonckheere Terpstra test was used for statistical analysis.

Results With increasing age there was no significant change in median haemoglobin (p-trend = 0.06) and erythrocyte count (p-trend = 0.06), while median reticulocyte count significantly decreased over time (p-trend = 0.01). MDRD clearance significantly decreased from the youngest age group to the oldest age category (p-trend < 0.001). Furthermore, there was a significant increase in EPO levels with increasing age (p-trend < 0.001).

Conclusion The findings of this study suggest that erythrocyte count and haemoglobin level remain stable througout live, whereas a decrease of the reticulocyte count was observed with increasing age. Furthermore, an increase in EPO level was found despite age related renal function loss. Two possible hypothesis are described. Nevertheless, further studies are needed to elucidate the mechanisms behind the observed increase of erythroipoietin and the maintenance of haemoglobin levels and erythrocyte counts in older persons.

Introduction

In old age, anaemia is believed to be caused by a decline in production of erythropoietin by age-related renal dysfunction. (1-3) Furthermore, it has been suggested that the ability of the kidney to secrete erythropoietin in response to tissue hypoxia declines with increasing age. (4) On the other hand, there is some evidence that erythropoietin levels in older subjects are similar to those of younger individuals. (5) Furthermore, one longitudinal study, in a cohort of 143 healthy adults aged 38-82 years without chronic kidney disease, found that erythropoietin concentration increases with advancing age. (6) We investigated the association between increasing age and serum levels of haemoglobin, erythropoietin (EPO), and renal function in randomly selected individuals in the range of 30-100 years.

Methods

Study population

From a study cohort, originally established to set reference values for various laboratory measurements for different age categories in the Bronovo Hospital, The Hague, The Netherlands, all persons aged 30 to 100 years were used for the current analysis. (7) The 275 subjects were consecutively enrolled into the study; 160 (58%) persons were employees from the Bronovo hospital, 41 (15%) subjects were living in nursing homes in The Hague and 74 (27%) persons were retrieved from the outpatient services from different general practitioners in The Hague. No inclusion criteria were applied. Exclusion criteria were pregnancy, diabetes mellitus, the use of oral contraceptives, vitamin- or iron supplements and oral anti-coagulants. From all participants a venous blood sample was drawn. The Medical Ethical Committee of Bronovo Hospital accorded the study and all participants provided informed consent for study participation.

Laboratory measurements

Haemoglobin, Mean Corpuscular Volume (MCV), erythrocyte, leucocyte, thrombocyte and reticulocyte count were measured on a LH750, Beckman Coulter Brea, Ca, USA. Serum EPO levels were measured using 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 than 6%. Creatinine was measured on a Synchron LX-20, Beckman Coulter. Creatinine clearance was estimated with the Modification of Diet in Renal Disease equation (MDRD).[2] Folic acid and vitamin B12 were determined in one batch using the Dual Count Solid Phase No Boil Assay (Diagnostic Products Corp, Los Angeles, California).

Statistical analyses

Differences in parameters over age categories were tested with Jonckheere Terpstra tests. Correlations between the various parameters were tested with Spearman's correlation coefficients. SPSS software (version 16.0.1, SPSS Inc, Chicago, Il) was used for all statistical analyses. P-values lower than 0.05 were considered statistically significant.

Results

Characteristics of study subjects in the various age groups are shown in Table 1. The study sample comprised 268 subjects, 141 (53%) women and 127 (47%) men. Median EPO level for subjects aged > 90 years was 12.4 mIU/ml, for subjects aged 76-90 years it was 12.5 mIU/ml, and for subjects in the younger age categories erythropoietin levels varied between 9.5 and 11.1 mIU/ml. The median haemoglobin level in subjects aged > 90 years was 7.9 mmol/L in all other age groups it was 8.7-8.8 mmol/L. Mean Corpsular Volume (MCV) was 91 fL in the subjects aged > 90 years and ranged between 90 and 93 fL in subjects in the other age catergories. Erythrocyte count for subjects age > 90 years was 4.2 x 10^9/L and for subjects age < 90 years erythrocyte count varied between 4.6 and 4.7 x 10^9/L. Reticulocyte count was 7.3% in the oldest age category and increased to 11.3 % in the youngest age category. Renal function, calculated by MDRD formula, was 64 ml/min/1.73m^2 in the oldest age category and 85 ml/min/1.73m^2 in the youngest age category.

Table 1. Characteristics of the Bronovo study population.

	30-45 year N=64	46-60 year N=77	61-75 year N=46	76-90 year N=32	>90 year N=10
Female, n (%)	33 (52)	40 (52)	25 (54)	18 (53)	5 (50)
Erythropoietin (mIU/ml)	9.5 (8.1-11.5)	9.7 (7.9-12.7)	11.1 (8.7-13.7)	12.5 (9.9-15.7)	12.4 (11.8-14.5)
Haemoglobin (mmol/L)	8.8 (8.3-9.3)	8.7 (8.2-9.4)	8.8 (8.5-9.2)	8.7 (7.7-9.2)	7.9 (7.6-8.3)
Erythrocyte (x 10^9/L)	4.7 (4.3-4.9)	4.6 (4.3-4.9)	4.6 (4.4-4.8)	4.6 (4.3-4.8)	4.2 (4.0-4.7)
Reticulocytes (%)	11.3 (7.3-13.9)	11.5 (8.1-15.3)	8.7 (7.3-12.5)	8.6 (7.7-12.5)	7.3 (5.5-10.3)
Creatinine (µmol/L)	83 (74-91)	86 (71-99)	83 (74-93)	89 (75-100)	98 (81-106)
MDRD (ml/min/1.73m^2)	85 (76-90)	76 (68-84)	73 (66-82)	66 (55-74)	64 (47-66)

Data presented are as median (IQR), unless otherwise stated.
Abbreviations: MDRD; Modification of Diet in Renal Disease equation.

A significant increase in EPO levels was found with increasing age (p-trend < 0.001) (Figure 1). Furthermore, with increasing age there was no significant change in median haemoglobin (p-trend = 0.06) and erythrocyte count (p-trend = 0.06), while median reticulocyte count significantly decreased over time (p-trend = 0.01). MDRD clearance

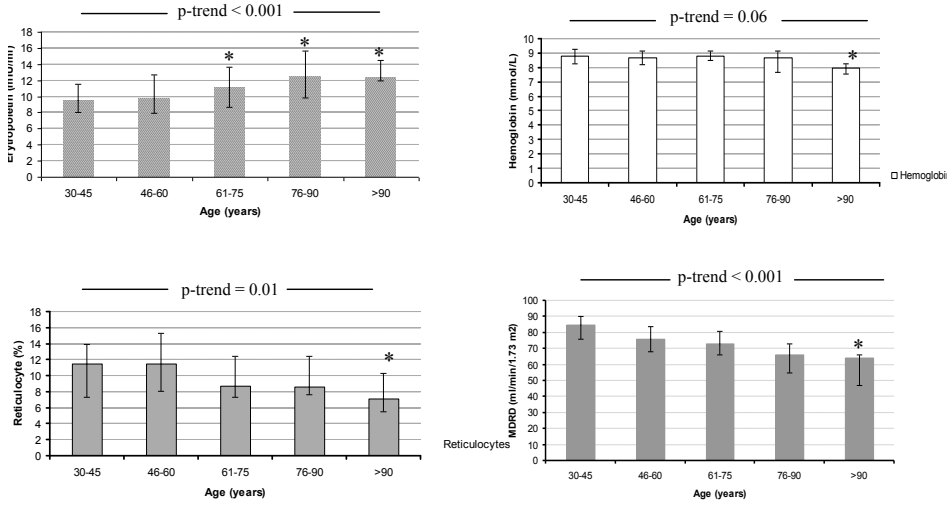

Figure 1. Erythropoietin, hemoglobin, reticulocyte levels and MDRD over age categories. All values are medians (IQR). P-values between the different age-categories were determined by Spearman's correlation coefficient, where the age-category 30-45 years was set as reference category (* P < 0.05). P-trend was calculated with Jonckheere Terpstra test.

significantly decreased from the youngest age group to the oldest age category (p-trend < 0.001). However, no significant difference between the different age categories was found for MCV, leucocyte or thrombocyte counts, or for folic acid and vitamin B12 levels (data not shown). Moreover, a significant correlation between erythropoietin level and reticulocyte count, p=0.012, was found.

Discussion

The most important finding in our study is the maintenance of haemoglobin levels and erythrocyte counts throughout life. Furthermore, we found that with increasing age reticulocyte count decreases and EPO levels increase.

These findings are comparable with an earlier French report (8) demonstrating that haemoglobin level and erythrocyte count are preserved at the same level during life. Furthermore, they found a significant lower reticulocyte count in people older than 65 years compared to younger individuals, while the EPO level increases at older age. Moreover, other reports showed that EPO levels tend to increase with age despite a decline in renal function. (3,6) Furthermore, in a large sample of community dwelling subjects of 65 years and older, only participants with severe stages of chronic kidney

failure (creatinin clearance of < 30 ml/min) had a significantly higher prevalence of anemia and significant lower erythropoietin levels. (4)

Although we found that reticulocyte count significant decreases, the total erythrocyte count remains stable with advancing age. This apparently conflicting phenomenon seems primarily to occur in order to maintain a stable haemoglobin level. There are several potential hypotheses (Figure 2). In our first hypothesis EPO has a central role. EPO, produced in the renal cortex, is an anti-apoptotic agent for the erythrocytic progenitors and after binding to the EPO-receptor it promotes the proliferation and differentiation of these cells to pro-erythroblasts and normoblasts through phosphorylation of a intracellular tyrosine-kinase pathway. (9) In addition, EPO protects newly formed erythrocytes from destruction resulting in a increased life span of these circulating cells. (10,11) The observed maintenance of a stable erythrocyte count during life despite a decrease of the reticulocytes may be explained by a prolongation of the erythrocyte survival time. This prolongation is most likely due to the observed increase in the EPO level. The question arises why increased EPO levels do not result in a sustained reticulocytosis. Therefore, it may be hypothesized that with increasing age haematopoietic stem cell become less responsive to EPO with diminished erythropoiesis. (12) This imminent anaemia leads to an increase in EPO production, but is inappropriate to overcome the diminished erythropoiesis. Due to the EPO-induced prolongation of the red cell survival the total erythrocyte count remains stable. In this first hypothesis the EPO increase is a secondary event and occurs despite an age-related decline in the renal function.

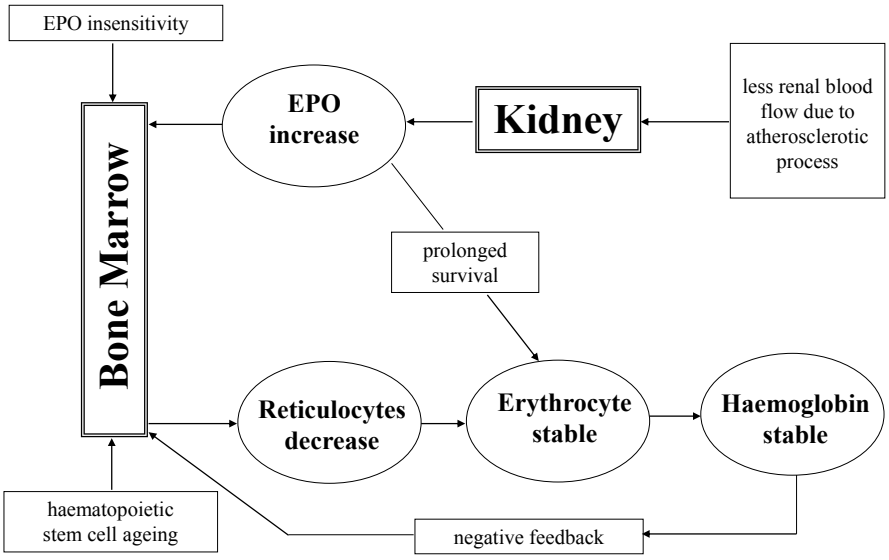

Figure 2. Possible pathophysiological mechanism as result of ageing kidney

Alternatively, it may be arqued that primarily EPO increases due to age-related local atherosclerosis within the kidney. (9) Because of the EPO-induced prolongation of the red cell survival, erythropoiesis must be down-regulated in order to maintain a stable erythrocyte count. Although it has been stated that after binding of EPO to the EPO-receptor, with subsequent activation of the erythroblasts, the EPO-receptor is downregulated. Clinical data indicate that continuous exposure to EPO does not lead to a decline in the reticulocyte count. (13,14)

Conclusion

In summery, in our first hypothesis EPO rises in order to compensate a diminished erythropoiesis and therefore prevent an imminent anaemia while in our second hypothesis erythropoiesis physiologically declines in order to avoid EPO-induced erythrocytosis. Nevertheless, irrespective of the underlying homeostatic mechanism, the events lead to stable erythrocyte count and haemoglobin level throughout life. Further studies are needed to elucidate the underlying homeostatic events.

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5

White blood cell count and C-reactive protein are independent predictors of mortality in the oldest old

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Abstract

Introduction White blood cell (WBC) count is, like C-reactive protein (CRP), a clinical marker of inflammation and predicts cardiovascular disease and mortality in middle aged populations. Limited data exist on the association between white blood cell count and mortality in the oldest old. Moreover, because CRP and WBC count are closely linked, it is not known whether WBC count and CRP are independent risk factors for mortality. We assessed the independent predictive value of WBC count and CRP levels in relation to mortality, both vascular and non-vascular, in very old subjects.

Methods A total of 599 women and men were evaluated longitudinally in the Leiden 85-plus Study. Blood samples and medical information were collected at age 85 and all subjects were visited annually until age 90 or death. Mortality risks were estimated with Cox-proportional hazard models.

Results Increasing WBC count was associated with an increased risk for all cause mortality (HR (95% Confidence Interval, CI) = 1.26 (1.15-1.38)) after adjustment for sex and smoking status. CRP levels were also associated with an increased risk for mortality (HR (95%CI) = 1.22 (1.10-1.35)). The association between increasing WBC count and mortality remained significant after adjustment for CRP levels (HR (95%CI) = 1.20 (1.09-1.33)), whereas also the relation between increasing CRP levels and mortality remained significant after adjustment for WBC count (HR (95%CI) = 1.17 (1.05-1.30)).

Conclusion Our results suggest that WBC count and CRP levels both independently predict mortality in the oldest old.

Introduction

White blood cell (WBC) count is a marker of systemic inflammation. Elevated levels of WBC count are associated with increased risk for morbidity and mortality in middle-aged populations. (1-13) Most studies have shown an independent association between high WBC count and increased risk for coronary heart disease and cerebrovascular events (1-3,7,11) and an increased risk for vascular mortality. (4-6,8-10,13) Only one study has shown that elevated WBC count was associated with increased non-vascular mortality. (12) Limited data exist on the association between WBC count and mortality in the oldest old.

Over the past decade C-reactive protein (CRP) has been shown to be a strong marker of systemic inflammation and a good predictor of vascular events and mortality. (14-17) Therefore, in clinical practice, CRP levels are nowadays considered to be a valuable marker of low-grade inflammation to predict mortality. Because the relation between WBC count and CRP levels has not been studied in detail (18), it is unknown whether by indentifying subjects with high levels of CRP, also subjects with high WBC count are identified. Moreover, it is also not known whether WBC count and CRP levels predict mortality independent of each other.

We investigated whether WBC count and CRP level are independent risk indicators for all-cause, vascular, and non-vascular mortalities in the oldest old, using a large population-based cohort of subjects aged 85 years who were followed for mortality over a period of almost 10 years.

Methods

Study population

The Leiden 85-plus Study is a prospective population-based cohort study of inhabitants of Leiden, The Netherlands. Between September 1997 and September 1999, all inhabitants of Leiden born between 1912 and 1914 (n=705) were contacted within a month after their 85th birthday. A total of 599 individuals (response rate 87%) agreed to participate (19). All participants were visited at their place of residence by medical staff and nurses. During this visit a structured face-to-face interview was conducted, a venous blood sample was obtained, and an electrocardiogram was recorded. The Medical Ethical Committee of the Leiden University Medical Center approved the study, and informed consent was obtained from all participants.

Mortality

All participants were visited annually until age 90 or death. After age 90 the population was followed up for mortality until February 2008 (censor date February 11, 2008). From the deceased participants the cause of death was obtained from the Dutch Central Bureau of Statistics. Only the primary cause of death on the death certificate was used in our analyses.
The International Statistical Classification of Diseases (ICD) codes used for vascular mortality are I00-I99. For non-vascular mortality all ICD codes except I00-I99 were used.

WBC cell count and CRP measurements

Of 562 participants hematological measurements like WBC count, hemoglobin, and thrombocytes were done at the time of recruitment at the Leiden University Medical Center with the fully automated system Sysmex XE-2100, TOA Medical Electronics, Kobe, Japan. The normal reference interval for the WBC count was set at 4.0 to 10.0 x 10^9/L. EDTA plasma was stored at −80°C. After recruitment, CRP was measured in one batch using the dual count solid phase no-boil assay (Diagnostic products Corp, Los Angeles, California).

Comorbid illness

Participants without comorbidity (good health status) were defined as having an absence of a history of stroke, myocardial infarction, diabetes mellitus, malignancy, chronic obstructive pulmonary disease, Parkinson's disease, dementia, hip fracture and arthritis at baseline. Arthritis was considered present when the medical history was positive for rheumatoid arthritis, osteo-arthritis or polymyalgia rheumatica. Presence of comorbid illnesses was obtained from participants' general practitioners by a semi-structured interview and by inspection of computerized records obtained from participants' pharmacies. (20) For participants living in a nursing home, the nursing home physician provided the necessary information. Apart from the medical records, diabetes, Parkinson's disease and chronic obstructive lung disease were also considered present when specific diabetes medication, anti-Parkinson drugs, or anti-asthmatics were prescribed. Furthermore, an electrocardiogram (ECG) was recorded and transmitted to a central ECG core laboratory where conventional interpretation was provided. Undiagnosed myocardial infarction was defined as the presence of a Q-wave myocardial infarction on the ECG that was unknown to the subject's general practitioner or nursing home physician. (21)

Statistical analyses

Since CRP levels were not normally distributed, CRP values were first log-transformed. Standardized values (per 1 SD increase) were used for comparability between the hazard ratios (HRs) from WBC and CRP. We assessed the standardized values by calculating

Z-scores. To calculate the Z-score we used the formula: (individual observation mean of study population)/ standard deviation study population. All associations of WBC and CRP separately were done with Cox proportional hazards model where WBC and CRP were entered as continuous variables in the models which were adjusted for sex, institutionalization and smoking status (Model 1). Model 2 includes both WBC and CRP levels together entered as a continuous variable , also adjusting for sex, institutionalization and smoking status. Hazard ratios (HR) were graphical depicted and indicate the change in risk by one standard deviation (SD) increase in WBC count or in CRP level. The interaction between WBC and CRP was assessed in one model with both WBC and CRP entered as continuous variables along with a cross-product term of the two continuous variables. The SPSS software (version 16.0.1, SPSS Inc, Chicago, Il) was used for all statistical analyses. P-values lower than 0.05 were considered statistically significant.

Results

Table 1 shows the baseline characteristics of the participants. Sixty-six percent of the participants were woman, 27% had no comorbidity, and 16% was a current smoker. The mean level of WBC count was 6.6 x 10^9/L. A total of 16 participants (3%) had a WBC count less than 4.0 x 10^9/L, 16 participants (3%) had a WBC count above 10.0 x 10^9/L. The mean CRP level was 7.6 mg/L, 85 participants (15%) had a CRP levels higher than the reference value of 10 mg/L. During the mean (SD) follow-up of 5.4 (3.1) years, a total of 452 (80%) participants died. Of those, 169 participants died of vascular mortality and 277 participants died of non-vascular mortality. From six participants the primary death cause was not known.

There was no significant association between sex and WBC (p=0.19), nor sex and CRP (p=0.08). Because there was a small difference in mortality rate between the sexes we nevertheless corrected for sex in all analyses.

The association between WBC count and CRP levels is shown in Table 2. Here, we show that the percent of participants who have concordant WBC/CRP tertiles is 45% (the diagonal in the table). The percent of the participants whose WBC tertile is lower than their CRP tertile is 26% and the percent whose WBC tertile is more than their CRP tertile is 29%. The correlation coefficient between WBC and CRP levels is 0.27.

The results of the association of WBC count and levels of CRP with mortality risks are shown in Figure 1. With an increase of one SD in WBC count we found a 1.26 (95% CI = 1.15-1.38) increased risk for all-cause mortality. Similar increased mortality risks were

Table 1. Baseline characteristics of the 562 subjects of Leiden 85-plus Study

	Living participants n =110	Deceased participants n= 452
Demographics		
Female, n (%)	87 (79)	285 (63)
Institutionalized, n (%)	10 (9)	93 (21)
Smoking status, n (%)		
Never smoker	67 (61)	67 (61)
Former smoker	28 (25)	28 (25)
Current smoker	15 (14)	15 (14)
No comorbidity*, n (%)	39 (36)	112 (26)
BMI (kg/m^2)	28.4 (4.2)	26.9 (4.5)
Bloodpressure (mmHg)		
Systolic	160.8 (17.2)	153.9 (18.7)
Diastolic	79.9 (8.6)	73.4 (9.7)
Laboratory results		
White blood cells (x10^9/L)	6.3 (1.4)	6.7 (2.3)
C-reactive Protein (mg/L)	4.0 (4.3)	8.4 (22.5)
Cholesterol status		
Total cholesterol (mmol/L)	5.9 (1.0)	5.7 (1.1)
Triglycerides (mmol/L)	1.8 (0.9)	1.5 (0.8)
HDL (mmol/L)	1.4 (0.3)	1.3 (0.4)
LDL (mmol/L)#	3.7 (0.9)	3.7 (1.0)

Data are presented in mean (SD), unless otherwise stated.

Abbreviations: BMI, Body Mass Index; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein.

* No comorbidity: no history at baseline of stroke, myocardial infarction, hypertension, hypercholesterolemia, diabetes mellitus, malignancy, chronic obstructive pulmonary disease, Parkinson's disease, dementia, hip fracture or arthritis.

LDL was calculated from total cholesterol, triglycerides and HDL levels.

observed for both vascular mortality (HR (95%CI) = 1.33 (1.18-1.51)) and non-vascular mortality (HR (95%CI) = 1.20 (1.05-1.37)) (Figure 1a). Furthermore, with an increase of

Table 2. Numbers of participants divided over tertiles of WBC count and CRP levels.

		Tertiles of CRP levels		
		1st	2nd	3rd
Tertiles of WBC count	1st	**97**	48	45
	2nd	65	**60**	55
	3rd	54	43	**94**

Abbreviations: CRP: C-reactive protein; WBC : white blood cell count. Bold values indicate concordant WBC and CRP tertiles

one standard deviation (SD) in CRP level we found a 1.22 (95% CI = 1.10-1.35) increased risk for all-cause mortality. Similar increased mortality risks were observed for non-vascular mortality (HR (95%CI) = 1.27 (1.11-1.45)). There was a tendency for an increased vascular mortality risk (HR (95%CI) = 1.15 (0.98-1.35) (Figure 1c).

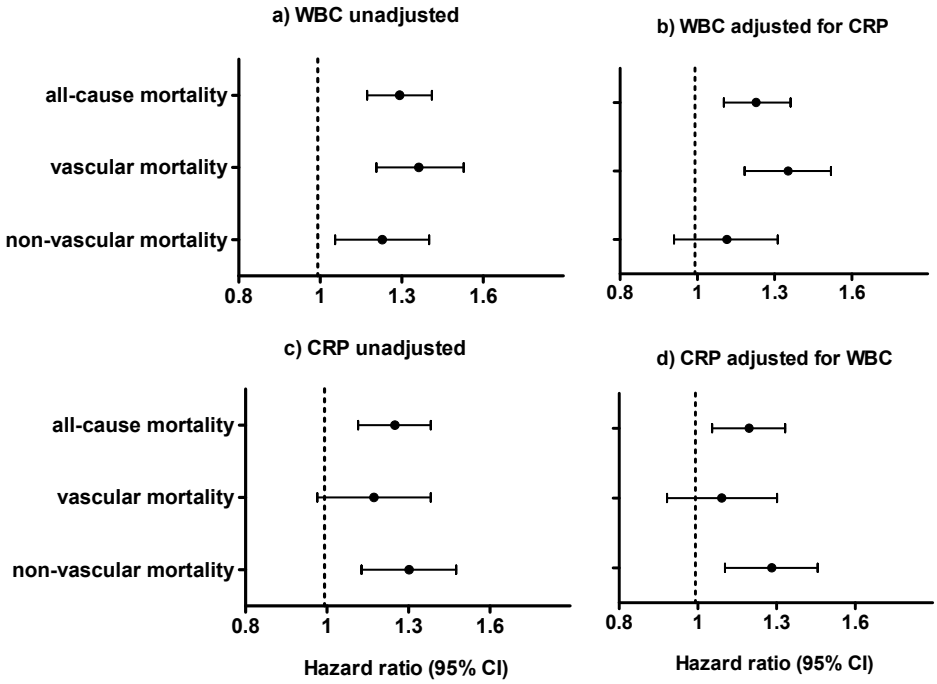

Figure 1: White blood cell (WBC) count, C-reactive protein (CRP) levels and mortality risk adjusted for sex, institutionalization and smoking status (never smoker, former smoker, current smoker). Data are presented as hazard ratio (95% confidence interval (CI)), per 1 *SD* increase. CRP levels were log-transformed.

There was no significant interaction between WBC count, CRP levels and all-cause mortality (p=0.56), and also not for vascular (p=0.86) and non-vascular mortality (p=0.48). Because there was no interaction between WBC count, CRP levels and all-cause and cause specific mortalities, we additionally adjusted the WBC associations with mortality for baseline levels of CRP (Figure 1). Adjustment for the level of CRP in the association between WBC count and all-cause mortality did also not materially change the results. The mortality risks for all-cause mortality (HR (95%CI) = 1.20 (1.09-1.33)) and for vascular mortality (HR (95%CI) = 1.32 (1.16-1.50)) remained significant after adjustment for CRP, whereas statistical significance was not reached for non-vascular mortality (HR (95%CI)

= 1.10 (0.94-1.28) (Figure 1b). Moreover, adjusting the association between CRP levels and mortality for baseline WBC count levels did not materially change the results (Figure 1d). This indicates that both WBC count and CRP level are independent predictors of mortality at old age.

To exclude the possibility that this association was driven by pre-existing disease we repeated all analyses stratified for presence and absence of comorbidity at baseline. The mortality risks of participants without comorbidity were highly comparable with the mortality risks in the group with co-morbidity which were all significantly higher than unity with increasing WBC counts or increasing CRP levels (data not shown). Moreover, we adjusted all analyses for the number of co-morbidities. The results did not materially change after adjustment (data not shown). Furthermore, we additionally adjusted all analyses for known cardiovascular risk factors, such as triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL) levels, systolic and diastolic blood pressure, and body mass index. Adjustment for these risk factors did not materially change our results (results not shown).

Discussion

We investigated the association between WBC count, CRP level and mortality in the oldest old. We found that both WBC count and CRP levels were independent predictors of mortality, in a dose-response manner. Stratification for comorbidity did not affect this association.

Our results confirm previous findings in middle-aged study populations that high WBC count predicts mortality independent of smoking and other co-morbid risk factors (1-4,6-10,12,13), and separately in a study of older men (age range 64-84 years) (5) and older women (age range 50-79 years). (15) As far we know it has not been described before that WBC count is also an indicator for mortality in the oldest old (above 85 years of age). It is unlikely that underlying diseases at baseline may have affected our observed associations because stratifying the analyses for comorbidity did not change our results.

In the twentieth century, WBC count was the most used marker for systemic inflammation. However, nowadays in clinical practice CRP levels are believed to be the most valuable diagnostic marker to predict mortality. (14-17) Several studies have shown that an increased CRP level in the elderly participants predicts an increased mortality risk, both vascular and non-vascular. (22-24) To our knowledge, the comparison of these both predictors, WBC count and CRP levels, in the relation with increased mortality has not

been described before. Here we show that both CRP and WBC count are independent predictors for mortality in the oldest old.

Although we found WBC predicting mortality, the pathophysiological explanation about the mechanism of elevated WBC count and mortality is not well understood. It is not known whether elevated WBC count is involved directly in the pathogenesis of vascular diseases or whether an increased WBC count is merely a risk indicator for other factors causing vascular damage. (25) For CRP this has been investigated by Mendelian randomization. (26-28) Participants carrying genetic variation within the CRP gene that determines a high CRP level did not have an increased risk for vascular mortality. (26-28) This indicates that high CRP plasma levels are not causally related to vascular disease but is merely an output of the vascular disease (reverse causality). Both WBC count and CRP levels are therefore risk indicators for an increased mortality risk.

Strength and limitations

The present study has several important strengths. First, these data are from a large, representative cohort of the oldest old and so far we know the relationship between CRP, WBC count and mortality has not been described in such a large representative cohort of this age. Second, because almost all our participants had a WBC count within the clinically defined normal range a potential contribution from reduced immune function or chronic infectious diseases is unlikely to have played an important role in the findings.

Potential limitations of this study must be considered. We used a single CRP and WBC count measurement to predict mortality. Multiple measurements over time may provide more accurate information for predicting future disease and therefore mortality. If so, with one single CRP and WBC measurement in our study cohort at baseline we may have underestimated the effect on mortality at old age. Moreover, although the number of WBCs can vary from day to day within one participant (29), a single measurement has previous been shown to predict risk for morbidity and mortality. Furthermore, the hazard ratios with WBC operated seemingly different from those associated with CRP for vascular and nonvascular mortality. However, the confidence intervals around the estimates are relatively wide. This makes it difficult to interpret whether there is a true difference. Finally, differential cell counts were not performed in the Leiden 85-plus Study, so we could not provide information about what types of leucocytes were more prone to be elevated with increased mortality risk at old age.

Conclusion

Our results suggest that that levels of WBC and CRP both are independently associated with mortality in the oldest old. Additional studies are needed to determine whether

interventions to decrease these markers can reduce the increased mortality risk. In clinical practice, it is necessary to pay more attention to the potential of WBC count as a predictor for mortality in the oldest old.

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No increased mortality risk in older persons with unexplained anemia

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Abstract

Introduction In older persons, anemia is associated with a number of unfavorable outcomes. In approximately 30% of older persons with anemia, the cause of the anemia is unexplained. We assessed the clinical differences between subjects with explained and unexplained anemia and investigated whether these subjects have different mortality patterns compared to subjects without anemia.

Methods In the Leiden 85-plus Study, an observational population-based prospective follow-up study in Leiden, The Netherlands, blood samples were drawn from 491 persons aged 86 years. The study population was divided in three groups: (1) no anemia (reference group, n=377), (2) explained anemia (iron deficiency, folate deficiency, vitamin B12 deficiency, signs of myelodysplastic syndrome, or renal failure, n=74), and (3) unexplained anemia, (n=40). Mortality risks were estimated with Cox-proportional hazard models.

Results Haemoglobin levels were significantly lower in subjects with explained anemia than in subjects with unexplained anemia ($p<0.01$). An increased risk for mortality was observed in subjects with explained anemia (HR 1.93, 95%CI 1.47-2.52), $p<0.001$), but not in subjects with unexplained anemia (HR 1.19, (95%CI 0.85-1.69), $p=0.31$). Adjusted analyses (sex, co-morbidity, MMSE, institutionalised and smoking) did not change the observed associations for both explained and unexplained anemic subjects.

Conclusion Older subjects with unexplained anemia had similar survival compared to non-anemic subjects. Increased mortality risks were observed in subjects with explained anemia compared to non-anemic subjects.

Introduction

The incidence and prevalence of anemia increase with age. (1,2) In older subjects anemia is associated with unfavorable events including death (3-6), dementia (7), cardiovascular diseases (8), and functional dependence. (9,10) Clinical investigation into the cause of anemia in older patients is often complicated by the co-morbidity characterising this patient group. Especially cognitive disorders and physical disabilities hamper reliable history taking and physical examinations, the cornerstones of medical practice. Therefore in clinical practice, the initial laboratory results are indicative for making the decision for further and often more invasive investigations in these patients. Since any procedure may lead to a deterioration of the current condition of the older patient invasive investigations should be limited to a minimum.

Chronic renal failure and iron-deficiency are the most common causes for anemia in older subjects (1,11,12), often caused by severe underlying diseases like atherosclerosis and gastro-intestinal malignancies, respectively. Other well known causes in clinical practice for anemia are folate- and vitamin B12 deficiency. The role of vitamin B12 deficiency as predictor for the presence of anemia in this population, however, is still subject to discussion. (13,14) Another serious cause of anemia in the elderly is myelodysplastic syndrome (MDS), a heterogeneous disorder characterized by ineffective haematopoiesis resulting in low peripheral blood counts. (15,16)

Several epidemiological studies have found that up to 30% of anemia in the older population is unexplained, even when extensive clinical information is available. (15,17,18) The clinical relevance of unexplained anemia is unclear. In the present study, we assessed clinical differences between explained and unexplained anemia in older subjects and investigated how mortality risks differ by type of anemia.

Methods

Study population

All data were derived from the Leiden 85-plus Study, a prospective follow-up study of subjects aged 85 years at baseline. At baseline, all individuals were living in Leiden, The Netherlands. There were no exclusion criteria for study participation. All subjects were visited at their place of residence where interviews took place and venous blood samples were drawn. Between 1997 and 1999, the Leiden 85-plus Study enrolled 599 subjects at baseline. (19) For the present analysis, we included 491 subjects aged 86 years. Of the 108 subjects that were not included in the present analysis, 47 died before the age of

86 years, 39 refused further participation, 16 refused blood sampling, and in 6 subjects no erythropoietin measurement was available due to technical problems. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the study and all participants provided informed consent for study participation.

Laboratory measurements

All blood samples were collected in sterile EDTA tubes. Measurements of haemoglobin, mean corpuscular volume (MCV) and erythrocyte count, white blood cell count and platelet count were done with a fully automated system (Sysmex XE-2100, TOA Medical Electronics, Kobe, Japan). Serum erythropoietin levels were measured using the enzyme immunoassay (EIA, Immulite 2500, Siemens Medical Diagnostics, Tarrytown, NY), which had a sensitivity of 1.2 mU/ml and a coefficient of variation less than 6%. Creatinine was measured according to the Jaffe method (Hitachi 747, Tokyo, Japan). Creatinine clearance was estimated with the Modification of Diet in Renal Disease equation (MDRD). (20) C-reactive protein (CRP) measurement was done in one batch using the Dual Count Solid Phase No Boil Assay (Diagnostic Products Corp, Los Angeles, CA, USA). Ferritin, folate, vitamin B12 were determined in one batch using the Dual Count Solid Phase No Boil Assay (Diagnostic Products Corp, Los Angeles, CA, USA).

Mortality

All subjects were followed up for mortality until 1 February 2008. For the deceased participants, the cause of death was obtained from Statistics Netherlands. Only the primary cause of death on the death certificate was used in our analyses. The International Classification of Diseases (ICD) codes I00-I99 were used for cardiovascular mortality. For non-vascular mortality, all ICD codes except I00-I99 were used.

Study groups

For the present study, the study population was divided in three groups: a group with no anemia (reference group), a group with a known cause for anemia (explained anemia) and a group with an unknown cause for anemia (unexplained anemia). Explained and unexplained anemia are two different identities; explained anemia, often due to chronic diseases or malignancies, is characterized by significant higher CRP, Interleukin-6 levels or higher erythropoietin levels. In persons with unexplained anemia, lower CRP, lower lymphocyte counts and EPO levels are found, assuming a totally different pathophysiological form of anemia.Anemia was defined according to the criteria of the World Health Organization (21) as haemoglobin levels lower than 12.0 g/dl (7.5 mmol/l) for women and 13.0 g/dl (8.1 mmol/l for men. Explained anemia was defined as anemia in the presence of iron deficiency (ferritin <20 ng/mL), renal failure (MDRD < 50 ml/min/m^2), vitamin B12 deficiency (<150 pmol/mL), folate deficiency (<7.0 nmol/mL), or

signs of myelodysplastic syndrome. Myelodysplastic syndrome was defined as anemia together with leucopenia (white blood cell count less than 3.5 x 10^9), or thrombopenia (platelet count less than 150 x 10^9). Unexplained anemia was defined as the presence of anemia with normal ferritin, vitamin B12, folate and creatinine concentrations.

Additional study parameters

Information on the presence of chronic disease (diabetes mellitus, chronic obstructive pulmonary disease (COPD), arthritis, myocardial infarction, heart failure, stroke and malignancy) was obtained from general practitioners, nursing home physicians and pharmacy records. (22) Cognitive function was measured with the Mini-Mental State Examination (MMSE). (23) All participants were interviewed about current and former smoking habits.

Statistical analyses

Data are presented as number (percentages) for dichotomous parameters and as median (interquartile range) for continuous parameters. Differences in laboratory values between anemic (both explained and unexplained) and non-anemic subjects (the reference group) were determined by Mann-Whitney U test. Mortality differences between anemic and non-anemic subjects were assessed with Cox regression analysis, adjusted for gender, co-morbidity, MMSE, institutionalisation, smoking and haemoglobin level. Mortality was visually depicted with Kaplan-Meier survival curves. Mortality differences between subjects with explained and unexplained anemia was additionally adjusted for haemoglobin level.

Since previous results from the Leiden 85-plus Study showed no association between vitamin B12 deficiency and anemia (13), we repeated all analyses excluding vitamin B12 deficiency from the explained anemic group.

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

Baseline characteristics

Baseline characteristics of the 491 subjects are presented in table 1. A total of 108 (22%) subjects were institutionalised in a nursing home or care home for older persons, 315 (64%) had one or more chronic diseases, 49 (10%) had a history of myocardial infarction, and 58 (12%) a history of stroke. A total of 131 subjects (27%) had renal insufficiency with an estimated glomerular filtration rate < 50 ml/min/1.73m^2 (MDRD). Of the 114 (23%) cases of anemia, 74 (65%) were explained and 40 (35%) were unexplained. Of the

Table 1. Baseline characteristics of the subjects at age 86.

	n=491
Women (%)	331 (67)
Institutionalized (%)	108 (22)
MMSE-score (median, IQR)	26 (22-28)
Current and/or past smoking (%)	231 (47)
Chronic diseases (%)	315 (64)
Malignancies (%)	92 (19)
Diabetes Mellitus (%)	73 (15)
COPD (%)	49 (10)
Arthritis * (%)	162 (33)
Renal insufficiency † (%)	131 (27)
Myocardial infarction (%)	49 (10)
Stroke (%)	58 (12)
Anemia (%)	114 (23)
Explained anemia (%)	74 (15)
Iron deficiency‡ (%)	13 (19)
Renal anemia ∫ (%)	42 (57)
Vitamin B12 deficiency ¶ (%)	11 (15)
Folate deficiency # (%)	4 (5)
Myelodysplastic syndrome**(%)	4 (5)
Unexplained anemia (%)	40 (8)
Follow-up time (years)	6 (3-9)

Continuous parameters are presented as median (interquartile range).
Categorial data are presented as numbers (percentages).
*Arthritis was defined as polymyalgia rheumatica, rheumatoid arthritis and osteoarthrosis.
†Renal insufficiency was defined as creatinine clearance <50 ml/min/1.73m^2 calculated by Modification of Diet in Renal Disease formula.
‡ iron deficiency was defined as ferritin <20 ng/mL; ∫ renal anemia was defined as having anemia and creatinine clearance <50 ml/min/1.73m^2; ¶ vitamin B12 deficiency was defined as <150 pmol/mL; # folate deficiency was defined as <7,0 nmol/mL; ** myelodysplastic syndrome vitamin was defined as anemia together with leucopenia (white blood cell count less than 3.5 x 10^9), and thrombopenia (platelet count less than 150 x 10^9)
Abbreviations: MMSE, Mini Mental State Examination; COPD, Chronic Obstructive Pulmonary Disease

74 subjects with explained anemia, 13 (19%) had iron deficiency, 42 (56%) had renal failure, 4 (5%) folate deficiency, 11 (15%) vitamin B12 deficiency and 4 (5%) myelodysplastic syndrome.

Anemia

Table 2 shows the hematological characteristics of the subjects with explained anemia, unexplained anemia and their counterparts without anemia. Hemoglobin levels were

significantly lower in the subjects with explained anemia than in the subjects with unexplained anemia (p<0.01) As expected, median erythropoietin levels were significantly higher in the subjects with anemia than in the subjects without anemia (9.6 mIU/ml) (p<0.01). However, median erythropoietin levels were similar in subjects with explained anemia and those with unexplained anemia, (11.5 mIU/ml vs 12.1 mIU/ml, p=0.88). Only in the group with explained anemia, CRP was significantly higher than in those without anemia (p<0.05). No differences in comorbid illnesses (malignancies, diabetes mellitus, COPD, arthritis, myocardial infarction and stroke), smoking, MMSE and institutionalisation were found between the explained and unexplained anemic groups (data not shown).

Table 2. Clinical characteristics of study participants by anemia status.

	Explained anemia (n=74)	Unexplained anemia (n=40)	Without anemia (n=377)
Hemoglobin (g/dL)	11.4 (10.4-11.8)**	11.7 (11.2-12.2)**	13.4 (12.8-14.1)
Hematocrit (%)	0.35 (0.32-0.36)**	0.36 (0.34-0.37)**	0.41 (0.38-0.43)
MCV (fL)	90 (86-93)**	91 (88-94)	91 (89-94)
Erythrocytes (x 10^9/L)	3.9 (3.6-4.1)**	3.9 (3.7-4.1)**	4.5 (4.3-4.7)
Leucocytes (x 10^9/L)	6.5 (5.1-7.6)	6.0 (5.0-7.1)	6.3 (5.3-7.5)
Platelets (x 10^9/L)	225 (172-272)	235 (194-282)	227 (191-267)
Erythropoietin (mIU/ml)	11.5 (8.8-17.2)**	12.1 (9.7-15.0)**	9.6 (7.5-12.8)
Creatinine (µmol/L)	105 (88-135)**	93 (79-100)	91 (80-106)
MDRD (ml/min/1.73m^2)	48 (37-66)**	62 (54-68)	58 (50-68)
Albumin (g/L)	39 (36-41)**	39 (36-41)**	41 (39-43)
CRP (mg/L)	6.0 (2.0-12.0)**	4.0 (1.0-8.0)	3.0 (0.0-7.0)
Ferritin (mg/L)	103 (37-229)*	93 (49-190)	118 (64-202)
Vitamin B12 (pmol/L)	282 (190-339)	302 (209-451)	256 (204-338)
Folic acid (nmol/L)	11 (9-14)**	13 (11-17)	13 (10-16)

Data are presented in median (interquartile range). P-value calculated by Mann-Whitney U test.
*=P<0.05, ** P≤ 0.01 compared to subjects without anemia.
Abbreviations: MCV; Mean Corpuscular Volume, CRP; C-reactive Protein.

Mortality risks

Survival differences between subjects with explained anemia, unexplained anemia and without anemia from age 86 onwards are visually depicted in Figure 1. An increased risk for all-cause mortality was found in the group of subjects with explained anemia (HR 1.93, 95%CI 1.47-2.52), p<0.001) compared with the group of subjects without anemia. Subjects with unexplained anemia had no increased risk for all-cause mortality compared with non-anemic subjects (HR 1.19, (95%CI 0.85-1.69), p=0.31). Adjustment

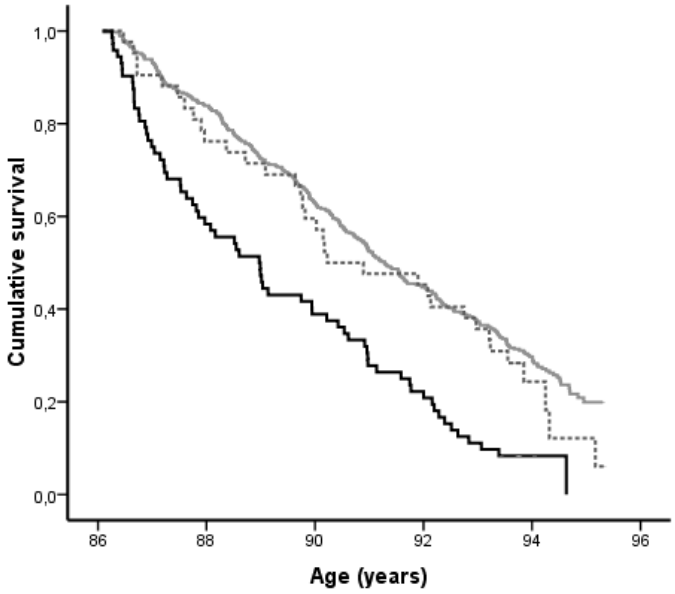

Figure 1. Survival of subjects with no anemia (n=377), explained anemia (n=74), and unexplained anemia (n=40) from age 86.

for sex, co-morbidity, MMSE, institutionalization and smoking did not change these associations for subjects with explained anemia ((HR 1.93 (95%CI 1.23-3.02), p =0.004) and for subjects with unexplained anemia ((HR 1.19, (95%CI 0.79-1.79), p=0.39). When we repeated all analyses excluding vitamin B12 deficiency from the explained anemic group, the results were not materially different.

Subjects with explained anemia had an increased mortality risk compared to subjects with unexplained anemia (HR 1.61 (95%CI 1.09-2.21), p=0.04). Adjustment for sex, co-morbidity, MMSE, institutionalisation, smoking and hemoglobin did not change this association. In this analysis, sex (HR 2.15 (95%CI 1.59-2.90), p<0.0001), hemoglobin level (HR 1.41 (95%CI 1.16-1.73), p=0.001), CRP (HR 1.02 (95%CI 1.00-1.02), p= 0.001) and MMSE (HR 1.07(95%CI 1.04-1.09), p=0.0001) were independently associated with mortality risk.

Furthermore, the all-cause, cardiovascular and non-cardiovascular mortality risks for participants with explained and unexplained anemia were studied. No statistically significant differences were found in cardiovascular mortality risk between the explained and unexplained anemic group (HR 1.83 (95%CI 0.90-3.74), p=0.09). However, no difference was found in non-cardiovascular mortality risk between these two groups (HR 1.36 (95%CI 0.81-2.32), p=0.24) (data not shown).

Discussion

The main finding of this study is that older subjects with explained anemia had an almost two-fold increased mortality risk compared to subjects without anemia, whereas in subjects with unexplained anemia no excess mortality was found. These findings possibly suggest that in older persons anemia is only relevant for survival when a clinical explanation for the cause of anemia can be demonstrated.

In our study sample, we found that in 37% of anemic subjects no clinical explanation for anemia could be found similar to earlier reports. Data from a non-institutionalised US population assessed in the third National Health and Nutrition Examination Survey (1988-1994) showed that unexplained anemia was present in one third of the older adults (>65 years) with anemia. (1) Another study reported that no evident cause for anemia in a representative sample of older Europeans (>65 years) was found in 36.8 % of the anemic participants. (18) Moreover, in a study conducted among subjects of a nursing facility almost one-third of the anemic subjects qualified as clinically unexplained. (17) Hence, our findings on the prevalence of explained and unexplained anemia in elderly subjects corresponds well with the prevalences reported in the literature.

We found a twofold increased mortality risk in older persons with explained anemia compared with the non-anemic subjects. We regarded subjects as having explained anemia when they met obvious clinical criteria as causes for anemia, i.e. iron-, folate-, vitamin B12 deficiency, renal failure, and myelodysplastic syndrome. Because of the design of our study, we were not informed about the specific underlying diseases causing these types of anemia. The reason for the observed difference in mortality between the subjects with explained anemia and unexplained anemia or without anemia is not obvious from our study. Between the three groups no differences in cause of death were found. It is tempting to speculate that persons with iron deficiency would have an increased mortality risk because of the presence of intestinal malignancy and that persons with a renal anemia would preferably die because of cardiovascular disease. However further analysis of our data failed to demonstrate such relationships, probably because of the small number of subjects.

Higher serum CRP levels were found in the group of subjects with explained anemia when compared to subjects with unexplained anemia and subjects without anemia. It may therefore be suggested that the underlying cause of the anemia is associated with a chronic inflammatory response which may contribute to the observed increased mortality. Since there was significant predominance of renal insufficiency and myocardial infarction in the explained group the observed elevated CRP may be the result of the inflammatory status due to underlying atherosclerotic disease.

In contrast to the group of subjects with known causes for anemia, the group with unexplained anemia had no increased mortality risk. To our knowledge, this is a new finding with potentially important clinical implications. It possibly suggests that when in older persons no cause for an anemia can be demonstrated by laboratory measurements, no further clinical investigations might be necessary. Anemia in general is associated with an increased mortality risk, also in the oldest old. (4-6) An explanation for the present observation is still subject to speculation. It can be hypothesized that in subjects with unexplained anemia, a relatively low hemoglobin level is part of their "normal" phenotype. However, the fact that erythropoietin levels in the subjects with unexplained anemia were similar to the level in the group with explained anemia, might suggest a physiological response to a relatively low hemoglobin level in these subjects. Based on the selection criteria used for this group, the absence of increased co-morbidity and the relatively normal CRP levels, it could be suggested that anemia in this group might be caused by impaired erythrocyte production due to an age-associated diminished hematopoietic stem cell proliferative capacity or, as suggested earlier, or by an one lineage MDS presenting as anemia without white blood cell or platelet features with a very low risk of further deterioration. (15) However, new studies are required to shed light on this clinically important finding.

Strengths and limitations

Our study contributes to the extensive discussion about the relationship between ageing and anemia. A strong point of our study is its prospective follow-up setting, permitting us to analyse survival of the oldest old subjects aged 86 years. A limitation of the study is the relatively limited sample size of subjects with explained and unexplained anemia. However, as mentioned, the prevalence of the subjects with unexplained anemia is remarkably similar to those reported in other studies. (1,17) Therefore we believe that our findings may have a high external validity. Another limitation is that we were only able to draw conclusions of subjects of 86 years or older who were still alive, more research have to be done on the impact of unexplained anemia and mortality of subjects younger than 86 years.

Conclusion

The present findings show that in contrast to older subjects with explained cause for anemia, older subjects with unexplained anemia based on initial laboratory measurements have no increased mortality risk compared to subjects without anemia, suggesting that further clinical and often invasive evaluation to the cause of anemia is possibly not necessary in this very old group of patients. This observation should be confirmed in other prospective observational studies and in dedicated intervention studies.

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7

Haemoglobin levels predicts length of hospital stay after hip fracture surgery in older patients

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Abstract

Introduction Treating anaemia in older patients who have undergone hip fracture surgery is to enhance functional recovery. The relationship between peri-operative haemoglobin levels and outcome after hip fracture surgery are controversial. We assessed whether higher haemoglobin levels predict length of hospital stay after hip fracture surgery in elderly subjects.

Methods A follow-up study in a historical cohort was performed in 317 patients aged 65 years old undergoing hip fracture surgery over the period 2004-2006 at the Leiden University Medical Centre. Linear regression analysis was used to assess the association between pre- and post-operative haemoglobin level and length of hospital stay after controlling for age and sex.

Results Anaemia after hip fracture surgery was present among 86% of the patients. Length of hospital stay after hip fracture surgery in elderly subjects with post-operative anaemia (10.7 days) was significant longer than in elderly subjects without post-operative anaemia (7.5 days, p =0.007). Post-operative haemoglobin levels and length of hospital stay were inversely related (p=0.013). The length of hospital stay was not related with pre-operative haemoglobin level.

Conclusion Higher postoperative haemoglobin levels predicts shorter length of hospital stay after hip fracture surgery in the elderly. A definitive randomized clinical trial has to demonstrate whether this association is causal.

Introduction

Hip fracture is a main cause of disability and death in older people, and numbers are expected to increase sharply in the near future. (1, 2) Poor functional recovery after hip surgery is related to loss of independence and long-term mortality. (3) Because hip fracture surgery is associated with considerable blood loss, some have reported that low peri-operative haemoglobin levels in older patients are associated with increased morbidity and mortality after hip surgery. (4-6) Functional recovery can be facilitated when treating anaemia. Moreover, Foss *et al* concluded that anaemia after hip fracture surgery in older patients impedes functional mobility in the early post-operative phase. (7)
Nowadays most hospitals in the Netherlands have a restricted transfusion policy after surgery due to associated risks with allogenic red blood cell transfusions, like infectious complications or acute lung injury. (8-11) A guideline that is frequently used in the Netherlands, advises red blood cell transfusion for patients over 60 years, if the haemoglobin level drops below 8g/dL or if anaemia is symptomatic. (12) This guideline is based on outcomes of studies that included patients younger than 60 years. (13) The haemoglobin threshold at which postoperative red-cell transfusion is warranted is controversial (14) and data of the benefits of red-cell transfusion after hip surgery in older subjects are scarce.
We assessed in older patients whether peri-operative higher haemoglobin level increases functional recovery and therefore shortens the length of hospital stay after hip fracture surgery. Therefore we performed a follow-up study in a historical cohort of older patients with hip surgery to determine whether pre- and post-operative haemoglobin levels predict length of hospital stay, which may provide provisionally evidence that a restricted transfusion policy has a (physical) cost.

Methods

Study population

Patients 65 years of age or older who were undergoing primary surgical repair of a hip fracture between 2004-2006 at the Leiden University Medical Centre by either trauma surgeons or orthopaedic surgeons were reviewed (n= 344). Patients with a re-operation within 1 month because of complications were excluded (n= 27). The total cohort included 317 subjects aged 65 years and over.
Peri-operative haemoglobin levels and length of hospital stay were retrieved from the hospital's patient information system. Length of hospital stay, the outcome measure for functional recovery, was defined as the difference between the day of admission and the day of discharge. The pre- and postoperative haemoglobin measurements reflected daily practice. The pre-operative haemoglobin value was measured. Furthermore, for

the postoperative haemoglobin level, the value measured one the day after surgery was used. If this was not available, the postoperative haemoglobin level measured in the recovery room was used. Anaemia was defined by the criteria of the World Health Organization.(15) The reference interval for haemoglobin concentration was set at 12-16 g/dL for women and at 13-18 g/dL for men. Anaemia was defined as a haemoglobin level of ≤ 12 g/dL for women and ≤ 13 g/dL for men. No in- or exclusion criteria were used, except an age of 65 years and over.
There was no permission required of the Leiden Medical Ethical Committee, because the research was done with historical data of patients receiving usual clinical care. No additional blood samples or questionnaires were needed.

Statistical analysis

The statistical analysis proceeded in various stages. First, descriptive statistics were performed using means and standard deviations (SDs) for continuous variables. Then, multivariate regression analysis was used to assess the association between pre- and post-operative haemoglobin level and length of hospital stay. Because length of hospital stay was not normally distributed, data were log transformed. Pre- and post-operative levels of haemoglobin were simultaneously entered in a linear regression model adjusted for age and sex. Then, results were transformed back to the original scale. All results on the association between haemoglobin and length of hospital stay are therefore presented as geometric means and corresponding 95% confidence intervals. All analyses were performed with SPSS for Windows (version 16.0, SPSS Inc, Chicago,Ill).

Results

The study population comprised of 223 women and 94 men, undergoing hip fracture surgery with a mean age of 80.4 years (range, 65-104). The clinical characteristics of the patients are shown in Table 1.

Table 1. Clinical characteristics of the study patients.

	All subjects
Age, years (range)	80.4 (65-104)
Male/female	94/223
Peri-operative transfusion, n (%)	136 (43)
Pre-operative haemoglobin (g/dL)	12.4 (1.7)
Pre-operative anaemia, n (%)	107 (33)
Pathologic/traumatic fracture	12/305

Data are presented as mean (SD), unless otherwise stated.

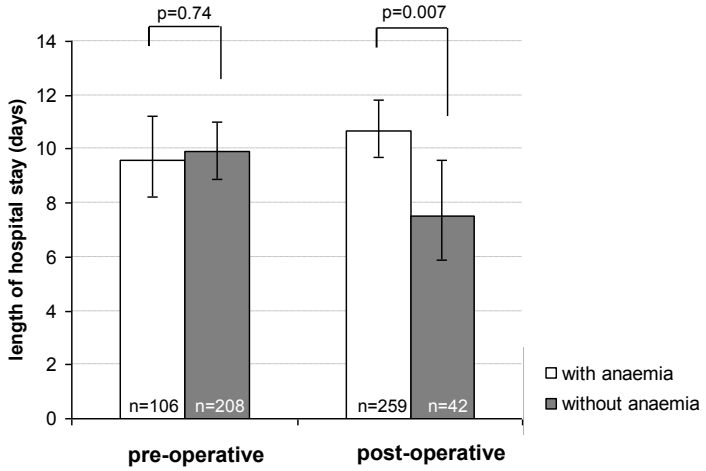

Figure 1. Pre- and post-operative anaemia of hip fracture surgery elderly patients in relation to length of hospital stay (mean and corresponding 95% confidence intervals), adjusted for age and sex.

The mean (SD) haemoglobin concentration before hip surgery was 12.6 (1.7) g/dL. After hip surgery the mean (SD) haemoglobin level was 10.4 (1.7) g/dL, the mean difference being 2.4 (1.4) g/dL. Before hip fracture repair anaemia was present in 33 % of the older patients. After surgery it was present in 86 %. At hospital discharge anaemia was still present in 68%. In-hospital mortality was less than 4 % (12/317 patients).

The mean length of hospital stay after hip fracture surgery in subjects with and without pre-operative anaemia was similar (9.6 vs 9.9 days, p=0.741) (Figure 1). In subjects with post-operative anaemia the mean duration of hospital stay was 10.7 days and in those

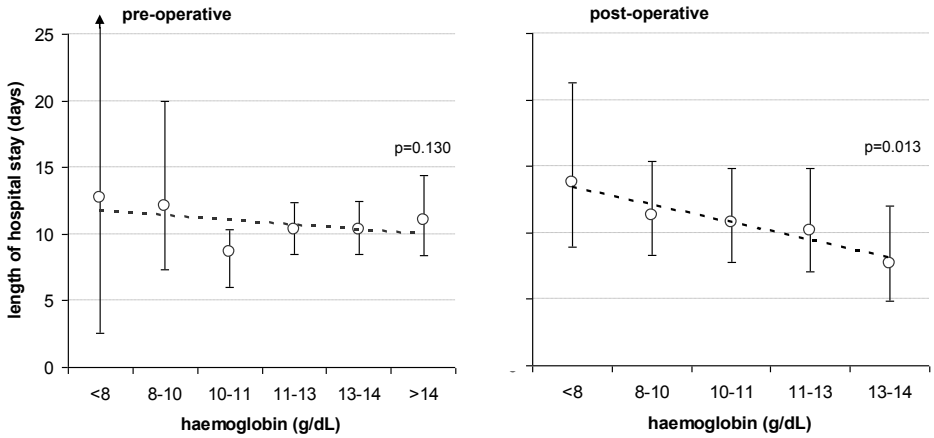

Figure 2. Categories of pre- and post-operative haemoglobin levels for hip fracture surgery in elderly subjects in relation to length of hospital stay, adjusted for age and sex.

without post-operative anaemia it was 7.5 days (p=0.007) (Figure 1). When pre- and post-operative haemoglobin levels were simultaneously entered into a linear regression model, adjusted for age and sex, pre-operative haemoglobin was not associated with length of hospital stay (p=0.130), while post-operative haemoglobin level was inversely correlated with a significant shorter hospital stay (p=0.013) (Figure 2). Moreover, subjects with no post-operative decrease in haemoglobin levels had a mean hospital stay of 9.2 days compared to 10.3 days for subjects with more than 3 g/dL decrease in haemoglobin (p=0.03).

Discussion

In this study we find that after hip fracture surgery, older patients with post-operative anaemia were significantly longer admitted postoperatively compared to those without anaemia after hip fracture repair.

The significant blood loss after hip fracture surgery in our study is in accordance with the literature. (16) This acute onset of post-operative anaemia in a large proportion of the patients has important clinical impact in older patients, not only on the cardiovascular system but also on the functional status of individuals, frequent in-hospital events, costs, resource utilization and even mortality. (4,6,17-19) Nevertheless, only few studies have evaluated the impact of acute anaemia on these outcomes in older subjects. (6,7,14,20) Since acute anaemia can cause such a wide spectrum of complications here we have chosen the length of hospital admittance as global outcome measure of this study, because it integrates all the relevant clinical events having an adverse effect on the functional recovery of the patient in a clinical setting.

The fact that older patients with post-operative anaemia stayed significantly longer in the hospital than patients without anaemia, may suggest that acute anaemia due to the blood loss has negative clinical impact on the post-operative functional recovery of these patients. The fact that 80% of all patients were anaemic after the surgical procedure results from the stringent blood management guidelines. (12) With incidence rates of less than 0.1 % for acute lung injury and of less than 0.01% for viral and bacterial infections due to red blood cell transfusions, the clinically important risks associated with these transfusions is low, and even lower when using leucocyte depleted red blood cell transfusions. (11,21,23) In contrast to the post-operative haemoglobin level, pre-operative haemoglobin was not associated with the length of hospital stay after hip fracture surgery.

Strength and limitations

This study has several limitations. First, we used a historical data collection, which did not permit measurement of haemoglobin levels at standardized time periods. Second, we could not control for differences in patient characteristics for example like co-morbid illnesses and institutionalization, which both influence hospital stay duration. However, the fact that we found no significant relation between pre-operative haemoglobin levels and hospital stay virtually rules out that pre-existing co-morbidity has influenced our results. Third, because of the permanent shortage of post-operative rehabilitation places outside the hospital after any type of surgery in our country, patients may have stayed longer in hospital than strict medical necessary, but this is likely to be randomly distributed over the study groups. Fourth, information about the timing of the given erythrocyte transfusions is lacking.Therefore we were not able to include the influence of transfusions on the length of hospital stay in our analysis. Furthermore, in our study population none of the patients received intravenous iron administration nor erythropoietin supplements.

Conclusion

In conclusion, this analysis generates the hypothesis that high post-operative haemoglobin values after hip fracture surgery in older patients is associated with shorter length of hospital stay. Length of hospital stay seems not related to pre-operative haemoglobin level. Our hypothesis requires testing in a randomized clinical trial. If a definitive prospective clinical trial demonstrates the same results and causality between higher post-operative haemoglobin levels and post-operative function and length of hospital stay after hip fracture surgery in older persons, current transfusion guidelines would have to be updated.

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8

Summary

Background

The aim of the research presented in this thesis was to study the physiology and clinical impact of changes in the triad renal function, erythropoietin production and hemoglobin levels in old age. The first part of this thesis focussed on the physiological aspects of age related decline in renal function and the relation with erythropoietin production and the maintenance of haemoglobin levels at old age. The second part of the thesis focussed on the consequences of changes in EPO, hemoglobin and other haematological parameters as predictors or indicators for mortality in older subjects. The underlying motivation for this research was to gain more insight in the possible consequences of physiological changes of kidney and bone marrow function during ageing with the objective to support the development of an evidence-based approach in clinical practice for these very old people.

Physiology of haemoglobin, erythropoietin and the kidney

Physiology refers to the normal function of organs, whereas "insufficiency" or " failure" is a pathological status. The knowledge of physiological processes taking place during ageing is of great importance because of the growing amount of the oldest old patients in daily practice. By 2050, oldest old people will account for one-fifth of all older persons globally. (1) In general, human organs are not affected uniformly by ageing. The hematopoietic system does not show any marked decline during ageing. (2) Anemia, low haemoglobin level, is therefore not a normal finding at old age. (3,4) But when present, with suggested prevalences of 40% in octarenarians (5), is associated with declined physical fitness, cardiovascular diseases and cognitive impairment. (6-9)

In contrast, renal function declines linearly with ageing. Chronic renal failure is predominantly a disorder of older people and predicts increased risk of all-cause and cardiovascular mortality. (10,11) However, within the kidney, the production of erythropoietin, the principal regulator of red blood cell production, seems not to be impaired by ageing. Furthermore, it is suggested that erythropoietin has independent of renal function a predictive value on survival. (12)

All these associations with mortality and the different organ changes at old age can only be understood when unravelling the physiological processes caused by ageing. Moreover, knowledge about physiology and survival could benefit patients well being and quality of life because it could improve clinical decision making in treatments for the oldest old.

Implications of age on estimating renal function

Since it is virtually unfeasible to measure GFR, for example by measuring inuline clearance in very old people, it is of great importance to calculate the most relevant estimate of GFR especially in the oldest old. The aim of the study described in chapter 2 was to study the effect of age on the –in clinical practice most used- formulae to compute renal function. The findings showed that the Cockroft-Gault formula, with inclusion of total body weight in the equation, is underestimating renal function at very old age in comparison with both MDRD and CKD-EPI formulae. Moreover, our study suggests that implementation of the newer CKD-EPI formula would raise the number of older individuals with chronic kidney disease. Based on these results it was concluded that estimation of renal function in very old persons can be facilitated by GFR equations, as long as clinicians realize that the precision of the formulae, C-G, MDRD and CKD-EPI, all have its own limitations.

The importance of adequate calculating renal function was further emphasized by the finding that in the oldest old subjects, impaired renal function (< 30ml/min/1.73m^2) is associated with an increased mortality risk. We found the MDRD equation is the most discriminative equation in predicting mortality in comparison with C-G and CKD-EPI in this age category. However, population based studies with 24-hour urine collections or with inulin or iothalamate infusions for the measurement of creatinine clearance are for practical reasons undoable in large numbers of very old subjects. Therefore, without a golden standard there will be always an uncertainty in the exploration of the most relevant equation(s) for calculating renal function in this age category. On the other hand, to find the most adequate calculated renal function for older people has important consequences such as in therapeutic decision making procedures, referrals to nephrologists and as predictor for survival.

Haemoglobin, EPO and renal function throughout life

In chapter 3 the results of the study of the association between increasing age and serum levels of erythropoietin, haemoglobin and renal function in healthy individuals aged 30-100 years are presented. The findings of this study, performed in the Bronovo Study, suggest that erythrocyte count and haemoglobin level remain stable throughout life, whereas a decrease of the reticulocyte count was observed with increasing age. Another important finding of our study was that we observed an increased erythropoietin level over time despite age related renal function loss.

Two possible hypotheses explaining these findings are described. In short, the most likely hypothesis is that EPO levels rise as a result of an EPO resistance bone marrow as a consequence of ageing. This higher EPO level protects newly formed erythrocytes from destruction and prolongs the erythrocyte survival time resulting in a stable total erythrocyte count. While with increasing age, haematopoietic stem cell become less responsive to EPO resulting in a decrease in reticulocyte count. The second hypothesis is that primarily EPO increases as a response to local hypoxemia due to age-related local atherosclerosis within the kidney. Because of the EPO-induced prolongation of the red blood cell survival, erythropoiesis must be down-regulated in order to maintain a stable erythrocyte count.
Nevertheless, the age related decline in renal function has no important consequences for the erythrocyte count and haemoglobin level throughout life, irrespective of the underlying homeostatic mechanism. Translating this interesting finding to clinical practice, is it important to realize that low erythrocyte count or low haemoglobin levels are not physiological, even in very old patients. The mechanism underlying the observed increasing EPO levels during ageing, suggesting progressive EPO resistance of the hematopoietic stem, should be subject to further investigation.

Haematological parameters in the oldest old

The study to access whether familial longevity can be attributed to sustained haematopoietic capacity compared to community dwelling older individuals is described in chapter 4. This study showed that familial longevity was associated with a benefit in survival, but no differences in haemoglobin level, leucocyte and thrombocyte count were found in comparison with subjects without familial longevity. Based on these results it was concluded that hematopoietic capacity cannot explain the survival benefit of those nonagenarians with familial longevity. Furthermore, it was found that all long living men and women with anemia had higher mortality rates than those without anemia. Therefore, lower haemoglobin levels in elderly subjects cannot be considered simply as a "result of ageing" but are in medical practice a reason for further clinical investigation with regard to the survival disadvantage.

Moreover, no relationship was found with low leucocyte or thrombocyte counts and mortality in subjects with familial longevity. The reported heritability estimates for hemoglobin, leucocytes and thrombocytes of respectively 0.37, 0.62 and 0.57 also indicate that hemoglobin levels are more volatile and therefore anemia reflects underlying morbidity more than leucopenia and thrombopenia. However, longitudinal studies

including bone marrow biopsy have to be performed to study the bone marrow reserve capacity of long-living subjects.

Independent predictors for mortality

In chapter 5 we have investigated the relation of white blood cell count (WBC) and also C-reactive protein (CRP) with mortality in the oldest old in more detail.
Elevated levels of WBC count and CRP are associated with increased risk for morbidity and mortality in middle-aged populations.(13-15) However, we show, within the Leiden 85-Plus Study, that both CRP and WBC count are also independent predictors for mortality in the oldest old in a dose-response manner. It is unlikely that underlying diseases at baseline have affected our observed associations because stratifying the analyses for comorbidity did not change our results.
Although we found WBC to predict non-vascular and vascular mortality in the oldest old, the pathophysiological explanation on the mechanism between elevated WBC count and mortality is not well understood. For example, for vascular mortality, it is not known whether elevated WBC count is involved directly in the pathogenesis of vascular diseases or whether an increased WBC count is merely a risk indicator for other factors causing vascular damage.[16] Our results suggest that increasing levels of WBC and CRP both are independently associated with increasing risk of mortality in the oldest old. Additional studies are needed to determine whether interventions to decrease these markers of inflammation can reduce the increased mortality risk. In clinical practice, it is necessary to pay more attention, besides CRP levels, to the potential of WBC count as a predictor for mortality in the oldest old, next to their potential to be a good predictor for infection.

Unexplained anemia: implications for survival?

In chapter 6 the association between unexplained anemia and survival is presented. Results showed that in our oldest old population 37% of the anemic subjects had anemia without an obvious clinical explanation. This relatively high prevalence of unexplained anemia in older subjects, means that for a substantial part of the elderly population no explanation will be found for their anemic status. Furthermore, it was shown that the older subjects with unexplained anemia had similar survival compared with subjects without anemia.

In contrast to the patients with unexplained anemia, an almost twofold increased mortality risk was observed in subjects with a clinical explanation for the anemia. Furthermore,

since unexplained anemia is a "diagnosis by exclusion", consensus must be reached in the exclusion criteria necessary to arrive at a diagnosis of unexplained anemia. In our study we regarded older subjects as having unexplained anemia when they did not met obvious clinical criteria as causes for anemia, i.e. iron-, folate-, vitamin B12 deficiency, renal failure, and myelodysplastic syndrome.

These results suggest that when in older persons no cause for anemia can be demonstrated, further clinical investigations is not necessary and allows for a watchful 'wait and see' strategy of these very old patients. Ideally this observation should be confirmed in prospective observational studies in older populations.

Haemoglobin level predicts length of hospital stay

Low haemoglobin levels and hip fracture are prevalent health conditions in elderly and often co-occur in elderly persons. The relationship between peri-operative haemoglobin levels and outcome after hip fracture surgery are controversial. The predictive value of haemoglobin level was studied in a historical cohort of older subjects with hip surgery in the Leiden Hipfracture Study and described in chapter 7. Post-operative haemoglobin levels and length of hospital stay were inversely related. Post-operative anemic subjects stayed significant longer in hospital after surgery. The length of stay was not related with the haemoglobin level before hip surgery. These results may suggest that in elderly patients acute onset anemia due to blood loss has negative clinical impact on the post-operative functional recovery of these patients. It seems that these data are a sufficient reason for a prospective clinical trial to demonstrate that post-operative anemia have negative impact on length of hospital stay and on functional mobility in the early post-operative phase. If that study shows the same results, than current transfusion guidelines in the Netherlands would have to be updated.

Nowadays most hospitals in the Netherlands have a restricted transfusion policy after surgery. The most used guideline advises red blood cell transfusion for patients over 60 years, if the hemoglobin level drops below 5 mmol/L or if anaemia is symptomatic. This guideline is based on outcomes of studies that included only patients younger than 60 years. Based on the present findings new prospective studies to investigate the benefits of red blood cell transfusion after (hip) surgery in older subjects are urgently needed.

Conclusion and future perspectives

Knowledge of physiological changes in renal function, EPO and haemoglobin level and their impact at old age are essential for clinicians especially those working with older patients. The results of the studies presented in this thesis provide more insight in the physiological aspects of age related decline in renal function and the relation with erythropoietin production and the maintenance of haemoglobin levels at old age. Furthermore, these results allow us to speculate about the predictive value of renal function, EPO and haemoglobin as markers of mortality in a clinical population of oldest old patients. Proper knowledge of these markers could contribute to increased attention of clinicians for the increased mortality risk of their oldest old patients. Furthermore, knowledge of these markers could be helpful in tailor made medicine, individual prognostication and decision making procedures, in the oldest old patients.

Three phenomena in oldest old subjects needs special attention for further study; the most adequate renal function equation, the value of EPO and the role of unexplained anemia.

Moreover, the challenge for calculating renal function at old age, is to understand the imperfections of the different renal function equations. It is of interest that in the oldest old the MDRD formula might be the best estimate and the newer CKD-EPI formula would raise the number of older individuals with chronic kidney disease in comparison with the MDRD formula, with consequences for therapeutic decision making procedures and resulting in more hospitalizations. Furthermore, referrals to nephrologists of increasing numbers of these oldest old patients could raise other dilemmas. The benefit of kidney biopsy or renal replacement therapy of those older people with their comorbidities must weighted against the complications and must add quality of life.

EPO levels in older subjects were found relatively low only when severe renal failure ($< 30 ml/min/1.73m^2$) was present. Further research need to be done to confirm these results in other populations of elderly people. If so, older subjects with severe renal dysfunction may be considered for erythropoietin substation therapy, which has been shown to improve quality of life in (pre)dialysis and cancer patients.

With regard to the best diagnostic approach in oldest old patient with anemia, the finding that subjects with unexplained anemia showed no excess mortality is of great interest. First, we found in line with the literature that older subjects with low heamoglobin levels are not “normal” and need further medical attention. Second, we found the exception in older subjects without an explanation for their anemic status. For this

unexplained anemic people, clinicians must realize that their low hemoglobin level has no impact on survival. It can be hypothesized that in subjects with unexplained anemia, a relatively low hemoglobin level is part of their "normal" phenotype. If so, than in the face of ageing, do those subjects have a survival advantage due to their beneficial rheology? Furthermore, might the unexplained anemia be caused by impaired erythrocyte production due to an age-associated diminished hematopoietic stem cell proliferative capacity or by an one lineage MDS presenting as anemia without white blood cell or platelet features with a very low risk of further deterioration and therefore without impact on survival? If confirmed in other prospective observational studies, further clinical and invasive evaluation to the cause of anemia is not necessary in this very old group of patients with unexplained anemia.

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9

Samenvatting

Inleiding

Met het ouder worden veranderen de fysiologische processen van het menselijk lichaam. De veroudering van verschillende weefsels en organen gaat gepaard met een toegenomen kwetsbaarheid van ons lichaam. De meeste functies zijn gewoonlijk vlak voor het dertigste levensjaar optimaal en nemen dan langzaam maar zeker af. Maar zelfs met dit verlies blijft de functionaliteit van de verschillende organen tijdens de rest van het leven op een voldoende niveau, aangezien onze organen over aanzienlijk meer functionele capaciteit beschikken dan het lichaam nodig heeft (functionele reserve). Hoewel de afname van de functionele capaciteit van tal van organen slechts weinig invloed heeft op de leefwijze van de mens, kan een dergelijk verlies van grote invloed zijn op de gezondheid, welbevinden en ook overlevingskansen. Veranderingen in de nierfunctie bijvoorbeeld kunnen grote gevolgen hebben voor de mate waarin bij ouderen sommige geneesmiddelen uit het lichaam kunnen worden afgevoerd.

Het eerste deel van dit proefschrift richt zich op onderzoek van de veroudering van de nierfunctie, op de capaciteit tot het produceren door de ouder wordende nieren van het hormoon erythropoëtine (EPO) en de aanmaak van hemoglobine door het beenmerg op oude leeftijd. Het tweede deel van dit proefschrift gaat over de consequenties van de achteruitgang in nierfunctie en het hebben van bloedarmoede (vermindering in hemoglobine) op oude leeftijd. Een belangrijke doelstelling voor dit onderzoek was inzicht te verkrijgen in de nierfunctie, erythropoëtine productie en het hemoglobine gehalte bij oudere mensen alsmede om te onderzoeken wat het effect van de genoemde determinanten is op de overlevingskans. Het achterliggende idee is om in de klinische praktijk gefundeerde adviezen te kunnen geven dan wel therapieën te kunnen initiëren of juist na te laten bij oudere patiënten met een verminderde nierfunctie, met verminderde erythropoëtine productie als mede bij ouderen met bloedarmoede.

Fysiologie : hemoglobine, erythropoëtine en nierfunctie

Belangrijk voor het begrijpen van ziekten bij oudere mensen is te weten hoe de verouderingsprocessen van de diverse orgaansystemen verlopen. Dit is met name van belang omdat het aantal ouderen de komende jaren fors zal toenemen en in 2050 zal een vijfde van de mondiale bevolking ouder zijn dan 85 jaar. (1)
De veroudering heeft verschillende invloed op de diverse orgaansystemen. Zo blijft bijvoorbeeld de beenmerg functie, welke onder andere zorgt voor de aanmaak van de rode bloedcellen, over de jaren heen redelijk intact. (2) Bloedarmoede op oudere leeftijd is dan ook niet een normale bevinding. Ondanks dat de gerapporteerde prevalentie

van bloedarmoede bij ouderen variabel is, wordt deze geschat op 40% in de algemene populatie van de oudste ouderen. (3,4) Het hebben van bloedarmoede is in algemene zin niet gunstig en wordt op oude leeftijd geassocieerd met afname van vitaliteit, functionele achteruitgang, cognitieve stoornissen en verhoogde sterftekans. (5,6) Van een ander belangrijk orgaansysteem, de nieren, is bekend dat de functie juist lineair afneemt met het vorderen van de leeftijd. Chronische nierziekte, zich uitend in een verminderde nierfunctie, komt veel voor op oudere leeftijd en is voorspellend voor zowel hart-en-vaatziekte als ook voor sterfte. (7,8) Ondanks de afname in nierfunctie lijkt het door de nieren geproduceerde hormoon erythropoëtine dezelfde spiegels te hebben op oudere leeftijd als op jonge leeftijd. EPO bevordert de vorming van rode bloedcellen en is recent ook, onafhankelijk van andere factoren, geassocieerd met mortaliteit op oude leeftijd. (9)
Voor het begrijpen en interpreteren van nierfunctie, EPO spiegels en bloedarmoede bij de oudere patiënt is de kennis van de gebruikelijke veroudering van deze organen onontbeerlijk. Ook wetenschap met betrekking tot overlevingskansen zou van waarde kunnen zijn bij de klinische besluitvorming bij deze oudste ouderen. Immers, bij beperkte overleving zal kunnen worden afgezien van belastende behandelingen van andere aandoeningen. Dit zal zeker bijdragen aan het welbevinden als ook aan de kwaliteit van zorg voor en kwaliteit van leven van (aller-) oudste patiënten.

Onderzoekspopulaties

Leiden 85-plus studie

Een belangrijk deel van het onderzoek beschreven in dit proefschrift maakt deel uit van de Leiden 85-plus studie. Deze studie is een longitudinaal onderzoek van een cohort Leidse ouderen van 85 jaar oud. Werving van deze ouderen vond plaats tussen 1997-1999. Alle inwoners van Leiden, ook als zij woonachtig waren in een verpleeghuis, werden kort nadat zij 85 jaar geworden waren uitgenodigd om mee te doen aan deze studie. Er werd hierbij niet geselecteerd op gezondheid of demografische kenmerken. (10) In het totaal hebben 599 ouderen deelgenomen aan dit onderzoek en dat is 87 % van alle potentiële deelnemers. Alle deelnemende ouderen werden thuis bezocht en geïnterviewd. Tenslotte werd bloed afgenomen en een electrocardiogram gemaakt. Gedurende 5 jaar werden vervolgens alle nog in leven zijnde deelnemers eenmaal per jaar bezocht.

Leiden Langleven studie

Voor de studie naar de associatie tussen hematologische parameters en overleving bij de oudste ouderen, werden de deelnemers van de Leiden Langleven studie geïncludeerd. In de Leiden Langleven studie namen 420 families deel, bestaande uit langlevende Kaukasische broers en zussen samen met hun kinderen en de partners van de kinderen. (11) De langlevende broers waren tenminste 89 jaar oud en de langstlevende zussen tenminste 91 jaar. Alle deelnemers bezochten het studiecentrum in Leiden alwaar ook bloed werd afgenomen.

Bronovo studie

Ter vergelijking van het hemoglobine gehalte, de EPO spiegels en nierfunctie van de oudste ouderen met jongere individuen werden de deelnemers van de Bronovo studie onderzocht. Het Bronovo cohort was eerder gebruikt om referentie waarden voor de verschillende bloedwaarden voor allerlei verschillende leeftijdsgroepen te bepalen. Dit onderzoek vond plaats in een perifeer ziekenhuis in 's-Gravenhage. In totaal werden er bij 275 random geselecteerde vrijwilligers in de leeftijd van 20-85 jaar bloed afgenomen, waarbij er niet werd geselecteerd op gezondheid of demografische kenmerken. (12) Alleen zwangere personen alsmede mensen met diabetes mellitus, gebruik van anticonceptiva, vitamine of foliumzuur suppletie dan wel orale antistolling werden uitgesloten van deelname.

Leiden Heupfractuur studie

Voor het onderzoek naar het effect van de hoogte van het hemoglobine gehalte op de opname duur na heupchirurgie werd de Leiden Heupfractuur studie gebruikt. In deze studie werden 344 patiënten na acute heupchirugie bekeken die ouder waren dan 65 jaar. Allen werden geopereerd in de periode 2005 tot 2006 in het Leids Universitair Medisch Centrum. Het enige exclusie criterium was patiënten met een re-operatie in verband met ernstige bloedingcomplicaties. Het totaal cohort bestond uit 317 ouderen. Hemoglobine waarden en opnameduur werden verkregen uit het patiëntinformatie systeem van het Leids Universitair Medisch Centrum.

Leeftijdsinvloed op nierfunctie

De glomerulaire filtratiesnelheid (GFR), het totale volume van urine wat in een gedefinieerde tijdseenheid gefilterd wordt door alle glomeruli van beide nieren bij mensen met een normale bloeddruk, is ongeveer 0,12 liter per minuut oftewel ca. 170 liter per dag. De GFR is de belangrijkste factor voor het inschatten van de nierfunctie. Aangezien het nagenoeg onuitvoerbaar is om bij de oudste ouderen de GFR te meten, bijvoorbeeld

door inuline toediening, is het zeer belangrijk om deze GFR adequaat te kunnen berekenen. In hoofdstuk 2 wordt de studie beschreven waarin het effect van leeftijd op de meest gebruikte formules om de nierfunctie te berekenen is onderzocht. De resultaten tonen aan dat de Cockroft-Gault formule, waarin het lichaamsgewicht is geïncorporeerd de nierfunctie onderschat bij de oudste ouderen in vergelijking met de MDRD en de CKD-EPI formules. Verder werden er aanwijzingen gevonden dat met implementatie van de nieuwe CKD-EPI formule het aantal oude mensen met chronisch nierlijden zal toenemen. Op basis van deze resultaten werd geconcludeerd dat het belangrijk is om in de klinische praktijk de voor- en nadelen van formules die nierfunctie berekenen te kennen en daarop in te spelen, zeker voor de oudste groep patiënten. Het belang van het adequaat calculeren van de nierfunctie wordt verder ondersteund doordat deze studie aantoonde dat een slechte nierfunctie bij oude mensen (< 30ml/min/1.73m^2) geassocieerd is met een verhoogd risico op overlijden. De MDRD formule voorspelt dit verhoogd overlijdensrisico het meest bij oude mensen. Echter meer onderzoek naar de meest adequate berekening voor nierfunctie voor de oudste ouderen is nodig.

Trias hemoglobine, erythropoëtine, nierfunctie en leeftijd

In hoofdstuk 3 worden de resultaten gepresenteerd van de studie naar de relatie van leeftijd op de spiegels van erythropoëtine en hemoglobine en de nierfunctie in gezonde vrijwilligers. De onderzoekspopulatie bestond uit deelnemers van de Bronovo Studie in de leeftijd van 30 tot 85 jaar. Het onderzoek toonde aan dat het hemoglobine gehalte en het aantal erythrocyten (rode bloedcellen) stabiel blijven over de jaren heen, in tegenstelling tot het aantal reticulocyten (jonge, onrijpe rode bloedcellen) welke dalen met toename in leeftijd. Verder werd ondanks achteruit gaande nierfunctie met toenemende leeftijd, verhoogde erythropoëtine spiegels gevonden op oudere leeftijd. Twee mogelijke hypotheses ten aanzien van deze bevindingen worden beschreven.

Kort gezegd is de meest waarschijnlijk verklaring dat het stijgen van de EPO spiegels de nieuwgevormde erytrocyten beschermd tegen afbraak en tevens hun overleving verlengd hetgeen resulteert in een stabiel aantal erythrocyten. Verder worden met het vorderen van de leeftijd de hematopoëtische voorloper cellen minder gevoelig voor erythropoëtine wat resulteert in een verminderd aantal reticulocyten. De hierdoor dreigende anemie leidt tot een stijging van de EPO productie.
De tweede hypothese is dat er primair een stijging van de EPO spiegel plaats vindt ten gevolge van lokale hypoxie in de nier door verminderderde nierdoorbloeding ten gevolge van arteriosclerose op oude leeftijd. Door de erythropoëtine geïnduceerde toename van de overleving van de erythrocyten moet de aanmaak van de rode

bloedcellen geremd worden om een stabiel aantal te bereiken. Het is van belang dat verder onderzoek gaat plaatsvinden naar het onderliggend mechanisme achter het gelijk blijven van het hemoglobine gehalte en het aantal erythrocyten op oudere leeftijd ondanks de stijging van het erythropoëtine.

Hematologische parameters bij oudste ouderen

De studie waarin gekeken wordt of bij ouderen met familiaire langlevendheid dit overlevingsvoordeel deels toegeschreven kan worden door toegenomen hematopoëtische (beenmerg) capaciteit staat beschreven in hoofdstuk 4. Het onderzoek toonde aan dat ouderen met familiare langlevendheid inderdaad een overlevingsvoordeel hebben, maar dat er geen verschillen waren in aantallen leucocyten, thrombocyten en het hemoglobine gehalte in vergelijking met gewone ouderen. Derhalve kan de verklaring van de langlevendheid niet gezocht worden in de hematopoëtische capaciteit. Verder bleek dat alle ouderen met familiaire langlevendheid met bloedarmoede een hoger overlijdensrisico hadden dan dezelfde ouderen zonder anemie. Geen relatie werd gevonden met lage aantallen witte bloedcellen en bloedplaatjes en overlevingskans in ouderen met familiaire langlevendheid. Gezien de verlaagde overlevingskans verdient het aanbeveling om bloedarmoede niet toe te schrijven aan leeftijd maar om verder onderzoek in te stellen naar de oorzaak van het ontstaan van de anemie. Meer studie in families met exceptionele langlevendheid en hematologische veranderingen is uiteraard nodig.

Onafhankelijke voorspellers van mortaliteit

De resultaten van de studie ten aanzien van de voorspellende waarden van witte bloed cellen en C-reactive protein (CRP) bij de oudste ouderen worden beschreven in hoofdstuk 5. Verhoogde waarden van witte bloedcellen en CRP is op middelbare leeftijd geassocieerd met een verhoogd risico op ziekte en sterftekans.(13-15) Deze studie laat zien dat ook bij de oudste ouderen zowel CRP als ook witte bloedcellen onafhankelijke voorspellers zijn voor sterfte. Het is onwaarschijnlijk dat onderliggende ziekten bij de start van de studie hieraan hebben bij gedragen, aangezien de resultaten hetzelfde bleven na correctie voor co-morbiditeit.

De pathofysiologische verklaring waarom verhoogde aantallen witte bloedcellen sterfte voorspellen is niet goed begrepen. Zijn deze verhoogde witte bloedcellen direct betrokken bij het ontstaan van vasculaire aandoeningen dan wel zijn deze witte bloedcellen

meer een risico indicator voor andere factoren die vasculaire schade veroorzaken? (16) Meer onderzoek naar het pathofysiologische mechanisme achter de verhoogde aantallen witte bloedcellen en hun rol bij de verhoging van de mortaliteit zou meer helderheid hierover kunnen opleveren. In de klinische praktijk lijkt het aan te bevelen om meer aandacht te besteden aan de potentie van witte bloedcellen als voorspeller van sterfte bij de oudste patiëntengroep.

Onverklaarde anemie en overlevingskans

In hoofdstuk 6 wordt de associatie tussen onverklaarde bloedarmoede en overleving beschreven. De resultaten laten zien dat bij onze oudste ouderen (85 jaar) in 37% van de mensen geen medische verklaring voor hun bloedarmoede werd gevonden. Deze relatieve hoge prevalentie van personen met onverklaarde bloedarmoede betekent dat onder een belangrijk deel van de oudere bevolking geen verklaring gevonden wordt voor hun bloedarmoede. Verder werd geconcludeerd dat de mensen met deze onverklaarde bloedarmoede gelijke overlevingskansen hadden in vergelijking met mensen zonder bloedarmoede. Deze resultaten zouden kunnen betekenen dat als bij oudere patiënten geen oorzaak voor de bloedarmoede kan worden gevonden, geen verregaande klinische onderzoeken hoeven plaats te vinden. Uiteraard zal deze bevinding verder onderzocht moeten worden, bij voorkeur in prospectieve observationele studies als ook in interventie studies. Overigens werd een twee keer zo hoog risco op overlijden gevonden in de groep ouderen met een bloedarmoede waarvoor wel een verklaring was gevonden in vergelijking met de ouderen zonder bloedarmoede en diegene met onverklaarde bloedarmoede. Het lijkt evident dat op basis van bovenstaande bevindingen verder onderzoek noodzakelijk is naar de implicaties van de onverklaarde bloedarmoede bij ouderen met als doel het aantal ziekenhuis bezoeken en belastende onderzoeken tot een minimum te beperken.

Hemoglobine voorspelt opnameduur na heupfractuur operatie

Bloedarmoede en heupfracturen zijn beiden veel voorkomende aandoeningen bij oudere mensen. De relatie tussen bloedarmoede en een heupoperatie vanwege een gebroken heup is controversieel. De voorspellende waarde van het hemoglobine gehalte rondom een heupoperatie werd onderzocht in een historisch cohort van oudere patiënten met een gebroken heup die in het Leids Universitair Medisch Centrum geopereerd werden en de resultaten zijn beschreven in hoofdstuk 7. Het hemoglobine gehalte dat bepaald wordt na de operatie is omgekeerd gerelateerd aan de opname-

duur in het ziekenhuis na een heupoperatie, terwijl het uitgangs-hemoglobine gehalte van voor de operatie geen enkele invloed heeft op de opname duur. Deze resultaten zouden kunnen betekenen dat bij oudere patiënten met acuut ontstane bloedarmoede ten gevolge van het bloedverlies bij de heupoperatie dit een negatieve invloed heeft op het post-operative beloop en herstel van deze ouderen. Aangezien er heden ten dage een strenge transfusie richtlijn geldt in ons land voor alle leeftijden, zou, indien onze resultaten worden bevestigd in grotere, prospectieve onderzoeken, de huidige richtlijn wat betreft ouderen moeten worden aangepast.

Conclusies en toekomstig onderzoek

Kennis met betrekking tot de fysiologische veranderingen door veroudering van de nierfunctie, EPO en het hemoglobine gehalte is essentieel voor klinici. De resultaten van de hier beschreven onderzoeken geven inzicht in de leeftijdgerelateerde verandering in nierfunctie en de relatie met erythropoëtine productie alsmede het handhaven van de hemoglobine spiegels op oudere leeftijd. Voorts kan er op basis van deze bevindingen gespeculeerd worden over de voorspellende waarde op overlevingskansen van de markers nierfunctie, EPO en hemoglobine bij de oudste ouderen. Het belang van deze kennis is een toegenomen alertheid onder medici op een verhoogd overlijdensrisico voor deze oudere personen. Verder kan deze kennis bijdragen aan de zogeheten "tailor made medicine" wat wil zeggen een behandelingsprogramma op het individu toegesneden.

Drie fenomenen bij de oudste ouderen die in dit proefschrift beschreven zijn verdienen in het bijzonder de aandacht voor toekomstig onderzoek: de meest adequate manier om de nierfunctie te berekenen, de waarde van EPO en de rol van onverklaarde anemie.

Het begrijpen van de tekortkomingen van de verschillende formules om de nierfunctie te berekenen op hogere leeftijd is een uitdaging. De MDRD formule lijkt de beste schatting te geven voor de oudste ouderen, terwijl het toepassen van de nieuwe CKD-EPI formule ten opzichte van de MDRD formule leidt tot een toename van het aantal ouderen met chronische nierinsufficiëntie, hetgeen consequenties heeft voor therapeutische besluitvorming en het aantal ziekenhuisopnames. Daarnaast kan een toename in verwijzingen van deze oudste ouderen naar de nefroloog leiden tot andere dilemma's. Het nut van een nierbiopsie of van nierfunctievervangende therapie bij deze patiënten met comorbiditeit moet opwegen tegen de complicaties. De eventuele meerwaarde ervan op de kwaliteit van leven moet hierin leidend zijn.

EPO spiegels in oudere individuen blijken relatief laag indien er sprake is van ernstig nierfalen (< 30ml/min/1.73m^2). Meer onderzoek is noodzakelijk om deze resultaten te bevestigen in andere populaties oudere patiënten. Indien dit het geval zou zijn, kan bij ouderen met ernstige nierfunctiestoornis, erythropoëtine substitutie therapie overwogen worden, wat haar waarde al heeft bewezen in het verbeteren van de kwaliteit van leven bij predialyse- en kankerpatiënten.

In lijn met de literatuur wijst ons onderzoek ook uit dat anemie bij ouderen niet "normaal" is en daarom ook aandacht verdient. In de benadering van de oudste ouderen met bloedarmoede is het belangrijk om te realiseren dat onverklaarde anemie niet is geassocieerd met meer sterfte. Theoretisch zou het zo kunnen zijn dat in deze groep patiënten met onverklaarde anemie, een relatief laag hemoglobine gehalte onderdeel is van hun "normale" fenotype. Hiervan uitgaande hebben deze mensen, in het licht van veroudering, wellicht een overlevingsvoordeel ten gevolge van hun gunstige reologie? Anderszins zou een onverklaarde anemie veroorzaakt kunnen worden door afgenomen erytrocyten productie ten gevolge van leeftijd gerelateerde capaciteitsafname van de haematopoëtische stamcel. Een myelodysplastisch syndroom (MDS) waarbij slechts een cellijn uitvalt (de erytrocyten) met hierbij een laag risico op verdere verslechtering van het ziektebeeld, kan ook een onverklaarde anemie zonder overlevingsnadeel verklaren. Als één van bovenstaande hypotheses in verder prospectief observationeel onderzoek bevestigd wordt, zal het in de toekomst niet meer nodig hoeven zijn om de oudste ouderen met onverklaarde anemie bloot te stellen aan klinische en/of invasieve evaluatie.

Uiteraard zal er naast verder onderzoek, blijvend aandacht besteed moeten worden om de vertaalslag tussen wetenschappelijk onderzoek en de dagelijkse klinische praktijk te kunnen blijven maken. Dit alles om de beste benadering voor onze oudste patiënten te kunnen faciliteren.

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Slotwoord

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Curriculum vitae

Jorien Margriet Willems werd op 12 juli 1973 geboren te Bilthoven. Na het behalen van het gymnasium diploma aan Het Nieuwe Lyceum te Bilthoven in 1991, begon zij in datzelfde jaar met de studie Biomedische Wetenschappen aan de Rijks Universiteit Leiden. Het propedeusejaar werd succesvol afgerond en in 1992 kon gestart worden met de studie Geneeskunde eveneens in Leiden. Het artsexamen behaalde zij in 1999. Vanaf 1999 tot 2004 was zij werkzaam als arts assistent in het Bronovo Ziekenhuis te 's-Gravenhage, alwaar zij in 2001 startte met de opleiding tot internist (opleiders Dr. R. Bieger † en Dr. J.W. van 't Wout). Voortzetting van de opleiding vond plaats in het Leids Universitair Medisch Centrum (opleiders Prof. dr. A.E. Meinders en Prof. dr. J.A. Romijn). In mei 2006 volgde de registratie tot internist en in 2007 werd de superspecialisatie ouderengeneeskunde afgerond. Van 2006 tot 2010 was zij werkzaam als staflid bij de afdeling Ouderengeneeskunde van het Leids Universitair Medisch Centrum (hoofd Prof. dr. R.G.J. Westendorp), waar zij begon met het in dit proefschrift beschreven onderzoek. Sinds 2010 is zij werkzaam als internist-ouderengeneeskunde in Noord-Brabant, thans in ziekenhuis Bernhoven te Uden.
Jorien Willems en Jeroen Vogelaar hebben samen twee kinderen: Jelle (2006) en Carlijn (2009).