Serum Erythropoietin and Aging: A Longitudinal Analysis

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OBJECTIVES: To determine the changes in serum erythropoietin with age in patients with and without anemia and to assess the importance of certain comorbidities on changes in erythropoietin level and the development of anemia.

DESIGN: Clinical history, hematological parameters, and serum erythropoietin levels were examined at 1- to 2-year intervals for 8 to 30 years.

SETTING: Baltimore Longitudinal Study on Aging (BLSA), National Institute on Aging.

PARTICIPANTS: One hundred forty-three BLSA participants.

MEASUREMENTS: Complete blood count and serum chemistries were performed at the time of each visit, and archived serum samples were used for erythropoietin level.

RESULTS: Although all subjects were healthy and without anemia at the time of initial evaluation, some developed chronic illness—most notably hypertension and diabetes mellitus. Erythropoietin levels rose significantly for the group as a whole, and the slope of the rise was found to be greater for those who did not have associated diabetes mellitus or hypertension. During the subsequent years, subjects who developed anemia but did not have hypertension or diabetes mellitus had the greatest slope in erythropoietin rise over time, whereas those with hypertension or diabetes mellitus and anemia had the lowest erythropoietin slope.

CONCLUSION: The increase in serum erythropoietin with aging may be compensation for subclinical blood loss, increased red blood cell turnover, or increased erythropoietin resistance of red cell precursors. It is suspected that, with very advanced age, or in those with compromised renal function (e.g., diabetes mellitus or hypertension), the compensatory mechanism becomes inadequate and anemia results. J Am Geriatr Soc 53:1360–1365, 2005.

Key words: erythropoietin; anemia; BLSA; aging

Anemia is a common condition in older persons. In community-dwelling older people, prevalence rates have been reported to range from 5% to 30%.[1–3] A recent comprehensive review of the National Health and Nutrition Examination Survey III data for individuals aged 65 and older indicated that 11.0% of men had hemoglobin levels below 13 g/dL and 10.2% of women below 12 g/dL.[4] In contrast, a recent survey of nearly 1,000 nursing home residents estimated the prevalence of anemia to be approximately 50% for both sexes.[5]

The question of whether the process of aging itself contributes to anemia has long been debated.[3] When examined closely, an etiology or associated condition for the anemia is usually uncovered, particularly if the anemia is moderate or severe,[6] but there remains a significant minority in whom no cause can be found. One proposed mechanism for the pathogenesis of unexplained anemia in older persons is inadequate erythropoietin production, but data on the effect of age on erythropoietin production from cross-sectional studies are limited and conflicting.[7–10] Although there is evidence that erythropoietin serum levels tend to increase with age, patients in whom the pathogenesis of anemia is unclear tend to have low levels of circulating erythropoietin.[11] This observation has also been reported for patients with diabetes mellitus, even without associated measurable renal insufficiency.[12]

Data on erythropoietin levels in nonanemic older persons have been inconsistent. Some have suggested that nonanemic older persons have higher erythropoietin levels than younger adults,[9] but other studies have failed to confirm these findings.[10–12] Erythropoietin levels measured in young adults are strikingly similar across studies, whereas erythropoietin levels for older subjects are found to be widely variable, not unlike other physiological measures in elderly populations. The data demonstrating higher erythropoietin levels in older persons were all in Japanese subjects and derived from cross-sectional observations.

The current article describes patterns of change over time in serum erythropoietin levels of participants in the Baltimore Longitudinal Study of Aging (BLSA) in relationship with changes in hemoglobin concentration, age, and the diagnosis of hypertension and diabetes mellitus. Based on these data, it is proposed that, with advancing age, greater levels of erythropoietin represent a physiological response required to maintain adequate red blood cell production. It was also hypothesized that, in very old subjects and in younger patients with diabetes mellitus or hypertension, this compensatory mechanism eventually becomes inadequate, leading to a greater risk of developing anemia.

METHODS

Participants

The BLSA is an intramural research program within the National Institute on Aging. Initiated more than 40 years ago, findings from this project have contributed greatly to our understanding of physiological changes that occur with normal aging (for a history of the BLSA, see[13]). Healthy volunteers aged 20 and older are enrolled in the study and participate in follow-up assessment visits approximately every 2 years. Currently, the study population has 1,400 active participants. An independent institutional review board approved the BLSA study protocol, and participants provided informed consent for all analyses included in this report.

A sample size estimate was undertaken based upon published reports of erythropoietin repeated measures and measurement variation. Calculations based upon fixed assumptions of five observations per person, an alpha level of 0.05, power of 0.80, and within-subject variance years from the first measurement of 3.16 projected a need for analysis of approximately 150 subjects.

Subjects were selected for this study if they had been active BLSA participants for at least 8 years, with a minimum of four visits, were not anemic (hemoglobin <12 g/dL for women and <13 g/dL for men), had not been diagnosed with renal insufficiency (defined as a 24-hour creatinine clearance of ≤60/mL/min), and had not received a transfusion or erythropoietin treatment within 6 months before the first sample used in this study. A total of 143 subjects with these characteristics were included in the study population. Of these, 77 were men, and all but four were Caucasian.

Measurements

With the exception of the serum erythropoietin determinations, all laboratory assays were performed at the time of patient visit within the clinical laboratories at the National Institute on Aging. Data extracted for this analysis included complete blood count (Coulter), erythrocyte sedimentation rate (ESR), and selected serum chemistries (serum creatinine, blood urea nitrogen, hemoglobin A1C, serum iron, total iron binding capacity, transferrin saturation, ferritin, vitamin B_{12} and folate levels, thyroid-stimulating hormone, and C-reactive protein).

A commercial laboratory (Covance, Princeton, NJ) measured erythropoietin in duplicate using an enzyme linked immunosorbent assay on aliquoted serum (frozen at the time of each visit ($-70°C$)).

The participant's primary physician defined diabetes mellitus and hypertension, which were provided by self-report and corroborated by current treatment (e.g., oral hypoglycemics, insulin, diuretics, or antihypertensives). BLSA participants complete a comprehensive health survey questionnaire at each visit, and all newly reported diagnoses are confirmed by communication with the primary physician. Anemia was defined as a drop of hemoglobin level below the World Health Organization's criteria for anemia (<12 g/dL for women and <13 g/dL for men). Also, for the purpose of this analysis, participants who sustained a drop in hemoglobin of 1.5 g/dL or more from the initial value and in whom the drop was maintained for at least two visits were considered to have developed anemia.

Statistical Evaluation

A linear mixed-effects (LME) analysis is an extension of the regression analysis that is appropriate for modeling longitudinal data characterized by correlated measurements and measurement observations that vary by number and time spacing across subjects.[14] Regression and the LME analyses were used in this study to analyze changes in hemoglobin and erythropoietin level over time, respectively, and changes in erythropoietin level associated with hemoglobin level over time for the entire group, in participants without diabetes mellitus or hypertension (Group 1) and in those who met predefined criteria for the diagnosis of diabetes mellitus or hypertension any time during the study (Group 2). The final form of the LME models used in the erythropoietin analysis is described in detail in an appendix that is posted at the Institute for Advanced Studies in Aging Website.[15]

RESULTS

Population Characteristics

Table 1 provides demographic information for the entire study population and in the most relevant subgroups used in the analysis.

Hemoglobin Levels with Age

In a large percentage of the individuals, hemoglobin concentrations remained fairly constant, and circulating levels of erythropoietin tended to increase. For example, Figure 1A depicts a typical pattern of a change over time in hemoglobin and erythropoietin in one of these subjects.

The overall trajectories of predicted hemoglobin over time for each individual participant are plotted in Figure 1B. There was a slight decline in hemoglobin concentration with age. This decline was demonstrated to be statistically significant using linear regression and LME analysis. In particular, the average rate of change over time in the hemoglobin level is -0.0376 g/dL per year ($P<.001$) using the regression analysis and -0.0552 g/dL per year ($P<.001$) using the LME analysis. Despite the gradual drop in level, 113 (79%) of the study participants maintained a hemoglobin concentration within the normal range, whereas 30 (21%) developed anemia as defined using World Health Organization criteria (<12 g/dL for women and <13 g/dL for men) or as a sustained drop in hemoglobin of 1.5 g/dL

Table 1. Participating Subjects

Subjects	n	Male/Female	Median Age at Entry (Range)	Median Hemoglobin Concentration (g/dL) at Entry (Range)	Median Erythropoietin Level (mIU/mL) at Entry (Range)
Entire group	143	77/66	62.0 (38.0–82.0)	14.7 (12.0–17.4)*	13.0 (6.0–47.1)
Group 1 (without hypertension or diabetes mellitus)	84	34/50	62.0 (50.0–81.0)	14.5 (12.0–16.5)*	12.4 (6.0–42.7)
With anemia	15	5/10	67.0 (54.0–76.0)	14.7 (12.0–15.5)	11.0 (6.0–23.5)
Without anemia	69	29/40	62.0 (50.0–81.0)	14.4 (12.5–16.5)*	12.8 (6.0–42.7)
Group 2 (with hypertension or diabetes mellitus)	59	43/16	62.0 (38.0–82.0)	14.9 (12.3–17.4)	13.3 (6.0–47.1)
With anemia	15	9/6	69.0 (52.0–82.0)	15.0 (12.3–17.4)	19.5 (6.0–28.1)
Without anemia	44	34/10	62.0 (38.0–78.0)	14.9 (13.1–16.5)	13.1 (6.0–47.1)

* One subject was missing a hemoglobin value.

from baseline (Table 1). Of the 84 subjects in Group 1 (no diabetes mellitus or hypertension), 15 developed anemia (18%), whereas 15 of the 59 subjects (25%) in Group 2 (diabetes mellitus and/or hypertension) developed anemia.

Erythropoietin Levels with Age

All subjects had a minimum of four paired hemoglobin and erythropoietin determinations, each separated by a minimum of 1 year. The overall trajectories of erythropoietin levels for each of the 143 participants are depicted in Figure 1C. By studying longitudinal changes in erythropoietin levels based on LME analysis, the predicted population average age increased over time, and the rate of change was 0.3760 mIU/mL per year ($P < .001$). The linear effects model predicted that all individuals' erythropoietin levels would increase by varying degrees over time.

Using the LME model on the entire study population, average longitudinal patterns of change in serum erythropoietin levels were estimated in accordance with hemoglobin concentration and time on study during the 31-year follow-up period. It was found that levels of erythropoietin were significantly and inversely related to hemoglobin concentration and increased significantly over time (Figure 2A). Hemoglobin concentrations were found to have a negative effect (-0.5137; $P = .09$) on predicted level of erythropoietin, and there was an interaction (-0.0602; $P = .01$) between follow-up time and hemoglobin concentrations, suggesting that the circulating average erythropoietin concentration increased and became progressively higher with lower hemoglobin levels.

An LME analysis fitted separately for participants without diabetes mellitus or hypertension (Group 1) and for those with diabetes mellitus and/or hypertension during the follow-up (Group 2) demonstrated patterns for each of the two groups (Figure 2B) that were similar to that of the entire group shown in Figure 2A. However, for each level of hemoglobin concentration, the initial erythropoietin levels were significantly higher in Group 2 than in Group 1 ($P = .003$), whereas the rate of change of erythropoietin over time was significantly less steep for Group 2 than Group 1 ($P = .03$).

An LME analysis was also performed considering four groups obtained by dividing Groups 1 and 2 into those who developed and did not develop anemia over the follow-up

period. First, the predicted patterns, based upon the model, revealed that there is a significant difference of 7.78 mIU/mL ($P < .001$) in initial erythropoietin levels in those who developed anemia during the course of study between Group 2 (18.88 mIU/mL) and Group 1 (11.09 mIU/mL). Within Group 2, the difference in initial erythropoietin level between those who developed anemia and those who did not was 3.66 mIU/mL ($P = .04$), indicating that those destined to develop anemia had higher starting erythropoietin values. This difference in initial erythropoietin level was not observed for those in Group 1 with or without eventual anemia.

As expected, in Group 1, the incremental rate of rise for erythropoietin over time was higher in participants who developed anemia than in those whose hemoglobin concentrations remained stable (0.50 mIU/mL per year vs 0.36 mIU/mL per year) (Figure 2C). In contrast, as seen in Figure 2D, the incremental rate of erythropoietin rise was lower in participants who developed anemia than in those who remained nonanemic (0.15 mIU/mL per year vs 0.24 mIU/mL per year). For those who developed anemia in Group 2, the slope of change over time in erythropoietin levels was not significantly different from 0. Figure 2D indicates that the longitudinal trends between Group 2 with anemia and without anemia are similar, with slowly increasing erythropoietin levels on average over time.

DISCUSSION

Erythropoietin is a renally secreted glycoprotein hormone, produced in response to tissue hypoxia.[16] Erythropoietin stimulates erythropoiesis, mainly through promoting the viability and differentiation of the colony-forming unit erythroid.[17] In response to anemia, healthy young subjects mount a several-fold increase in erythropoietin levels.[18]

Although a decline in erythropoietin response has been implicated in the pathogenesis of anemia under a variety of circumstances, including advanced age, little is known about normal changes in erythropoietin secretion with age. Using data collected serially in a group of 143 BLSA participants followed for 8 to 31 years, a significant rise in erythropoietin level over time was found. This was true for those who maintained a normal hemoglobin over time as well as for those developed anemia. Furthermore, for those

who developed hypertension or diabetes mellitus, the rise in erythropoietin, although present, was less marked than that observed in those not affected by these conditions. It is conceivable that this blunting reflects an age-acquired renal impairment from the underlying disorders. The magnitude of the age-associated mean increment in erythropoietin was consistently maximal in nondiabetic, nonhypertensive participants who developed anemia, whereas in those with diabetes mellitus or hypertension, a significant change in erythropoietin level did not accompany the onset of anemia. These findings suggest that, with age, increased erythropoietin production is necessary for the maintenance of hemoglobin concentration and that this compensatory mechanism is less efficient in individuals with diabetes

mellitus or hypertension, who show a diminished erythropoietin response.

In addition, individuals who were destined to develop anemia later in life, particularly those with diabetes mellitus or hypertension, had higher levels of erythropoietin at the baseline evaluation (see Figure 2B). Although the levels are within the "normal" range, a high-normal erythropoietin level in a young or middle-aged nonanemic adult may be a harbinger of evolving glucose intolerance or hypertension, a finding that warrants additional investigation.

Furthermore, it is unclear whether aging is associated with shortened red blood cell survival. If this were the case, higher erythropoietin levels might be an expected finding, provided that age or coexisting disease has not impaired the kidney's capacity for producing and secreting erythropoietin.

Inadequate erythropoietin production as a contributing factor to the development of anemia has become increasingly recognized in the context of specific medical conditions. A blunted endogenous erythropoietin response to anemia has been observed in patients with cancer,[19] human immunodeficiency virus,[20] rheumatoid arthritis,[21] and inflammatory bowel disease[22] and in premature infants.[23]

Studies in aged mice have demonstrated a diminished erythropoietic response to anemia or hypoxia.[24] Similarly, most,[7,10,11,25] but not all,[12] human studies have suggested an inadequate erythropoietin response to anemia in older persons. However, methodological concerns have complicated these studies, particularly in defining appropriate statistical analyses and relevant control groups. Thus, it is unclear whether an observed diminished erythropoietin response is a feature of aging itself or the associated comorbidities existing in the populations studied.

The data suggest two novel conclusions. First, in patients who do not develop anemia, higher levels of erythropoietin are required to sustain normal hemoglobin concentrations as a person ages. This is possibly due to an evolving resistance or diminished responsiveness of the erythropoietin sensitive progenitor cells. Second, in persons who develop anemia, some processes interfere with the
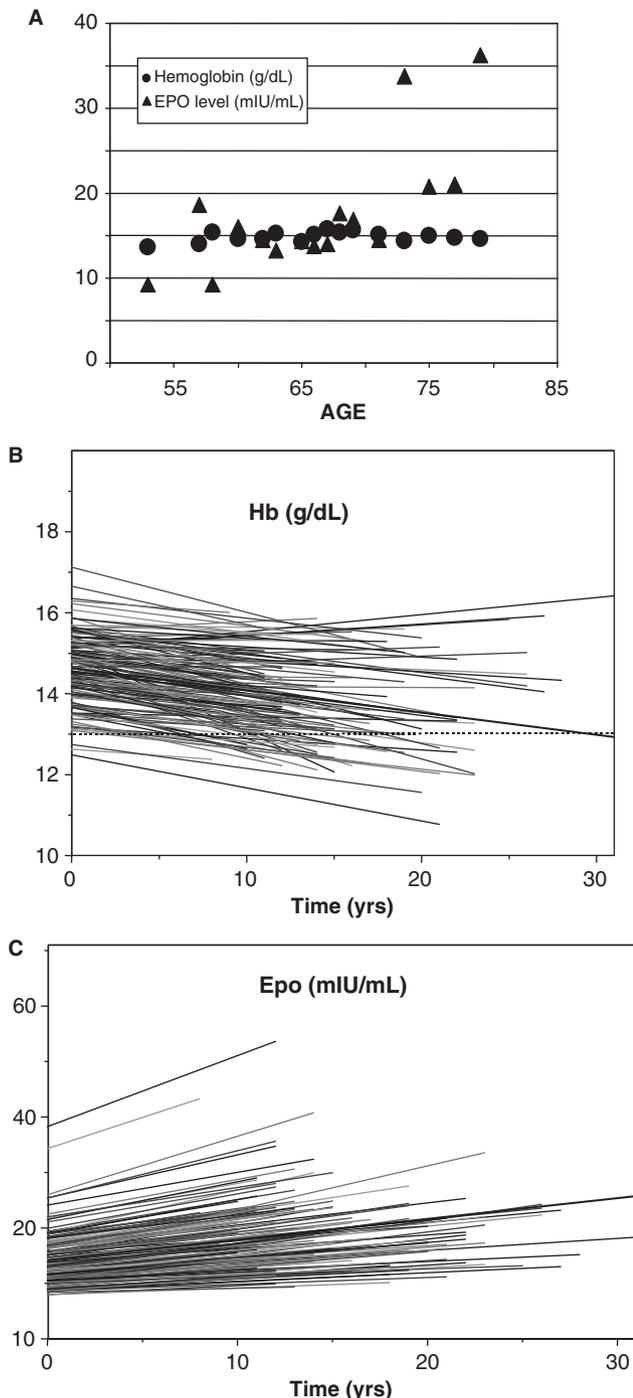

Figure 1. Hemoglobin (Hb) and erythropoietin (epo) changes with age.

A. Longitudinal analysis of a single representative participant. Hemoglobin concentration remained constant over the nearly 31 years of observation, but erythropoietin levels were shown to increase with advancing age.

B. Hemoglobin levels obtained on all 143 subjects on a minimum of four occasions, each separated by approximately 2 years. Each of the thin lines indicates the predicted hemoglobin concentration for each individual, the bold line represents the population average change over time, and the dotted line is a reference to the World Health Organization criteria for anemia for men (13 g/dL).

C. Serum erythropoietin levels were measured on a minimum of four occasions, each separated by approximately 2 years. Each of the thin lines indicates the predicted change in erythropoietin for each individual. The bold line represents the population average change over time.

(Details of the limited-mixed effects model used in this analysis can be found in an appendix at the Institute for Advanced Studies in Aging Web site: www.iasia.org.)
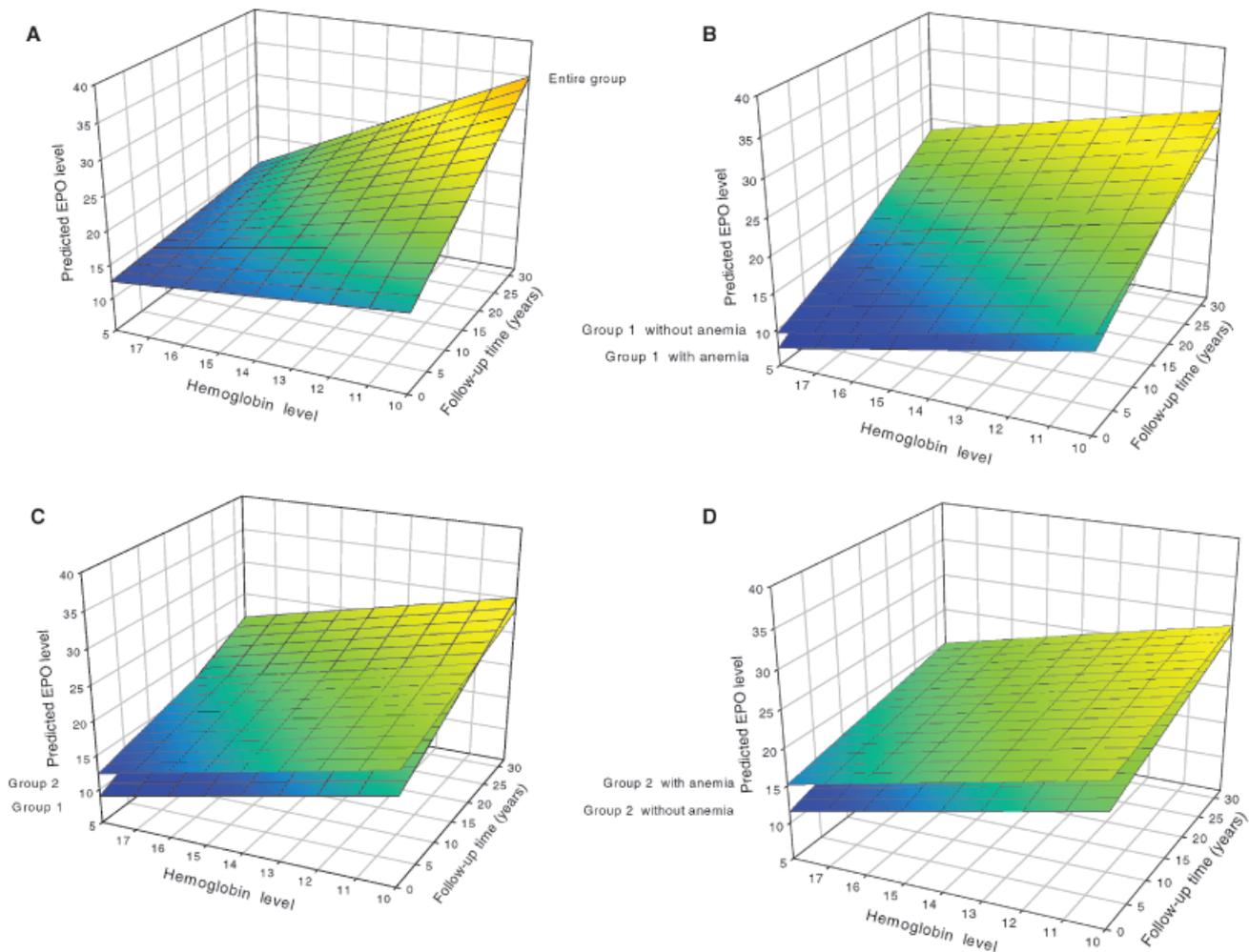
Figure 2. Predicted erythropoietin levels from the linear mixed-effects model as a function of hemoglobin level (g/dL) and time (years): (A) for the entire sample, (B) for the two groups, and (C) for Group 1 with and without anemia and for Group 2 with and without anemia.

production or secretion of erythropoietin to sustain a normal hemoglobin concentration.

Several mechanisms may account for an inadequate erythropoietin response in older persons. Cytokine dysregulation may reduce erythropoietin production or bone marrow responsiveness,[26] occult interstitial renal dysfunction without a change in glomerular filtration may diminish production,[10] or the set point for secretion may be lowered. It is the authors' hypothesis that aging is associated with a decline in the capacity to produce erythropoietin by a mechanism that is distinct from impaired renal excretory function. Certain diseases or disease processes, such as diabetes mellitus or hypertension, are known to negatively influence renal tubular function and may accentuate the impairment in erythropoietin secretion.

Renal excretory function declines with age, and this seems to be more notable for those with hypertension or diabetes mellitus.[27] Similarly, the capacity to produce or secrete erythropoietin may decline with age and diseases, independent of other renal excretory function, and cause the development of anemia in patients with normal serum creatinine. An increased production of erythropoietin with age has been described, and it has been proposed that, in some, the higher level of production is not sustainable, accounting for or contributing to the development of anemia in these individuals.

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