Anemia in Hemodialysis Patients: Variables Affecting this Outcome Predictor

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Abstract. Despite the prevalent use of recombinant human erythropoietin (rhEPO), anemia is a frequent finding in hemodialysis patients. The goal of this study was to evaluate the impact of anemia on patient survival and characterize the determinants of hematopoiesis that may be amenable to therapeutic manipulation to enhance rhEPO responsiveness and reduce death risk. Patient characteristics and laboratory data were collected for 21,899 patients receiving hemodialysis three times per week in dialysis centers throughout the United States in 1993. Hemoglobin concentrations (Hb) ≤80 g/L were associated with a twofold increase in the odds of death (odds ratio = 2.01; $P = 0.001$) when compared with Hb 100 to 110 g/L. No improvement in the odds of death was afforded for Hb >110 g/L. Using multiple linear regression, variables of rhEPO administration (rhEPO dose and percentage of treatments that rhEPO was administered), variables of iron status (serum iron, transferrin saturation, and ferritin), variables of nutritional status (serum albumin and creatinine concentration), and the dose of dialysis (urea reduction ratio) were found to be significantly associated with hemoglobin concentration ($P < 0.001$). Age, race, and gender were also found to be significantly associated with hemoglobin concentrations ($P < 0.001$). From this report, the following conclusions may be made. (*1*) Anemia may be predictive of an increased risk of mortality in some hemodialysis patients. (*2*) Hemoglobin concentrations >110 g/L are not associated with further improvements in the odds of death. (*3*) Laboratory surrogates of iron stores, nutritional status, and the delivered dose of dialysis are predictive of hemoglobin concentration. Whether manipulation of the factors that improve anemia will also enhance the survival of patients on hemodialysis is unknown and should be evaluated by prospective, interventional studies. (J Am Soc Nephrol 8: 1921–1929, 1997)

Several routine laboratory variables have been identified as predictors of survival in patients undergoing maintenance hemodialysis (1–3). On the basis of logistic regression models of outcome, surrogates of nutrition (such as the serum albumin and creatinine concentrations) and measures of delivered dose of dialysis (such as the fractional reduction of urea, or urea reduction ratio [URR]) are the principal predictors of survival for dialysis patients (1–3). Other factors have also been described but have received less attention because they were found to have a less significant statistical impact on patient survival. However, these other variables may be more amenable to interventions and, as a result, merit scrutiny. Anemia is one of these factors. Dialysis patients with a hematocrit level of <20% have a probability of death that is one and a half to three times greater than patients with a normal hematocrit level (2,3). The correction of the anemia may be a relatively simple means of improving the probability of survival for dialysis patients.

The clinical introduction of recombinant human erythropoietin (rhEPO) in the last 6 yr has allowed therapeutic manipulation of the hematocrit level in hemodialysis patients. However, despite the prevalent use of rhEPO, anemia remains a common finding in hemodialysis patients. As reported by the U.S. Renal Data System for patients on hemodialysis in 1993, the mean hematocrit was 30.5% despite a mean rhEPO dose of 3923 U three times weekly (4). Treatment with rhEPO is expensive, adding approximately 30% to the cost of a dialysis treatment. Therefore, strategies have been proposed to enhance the erythropoietic response to rhEPO. These strategies include subcutaneous instead of intravenous rhEPO administration (5), the routine administration of parenteral iron supplements (6), or both. Despite the reported efficacy of these interventions, they have not been widely implemented because of patient and physician unacceptance, and fear and intolerance of their side effects. Clinical practice guidelines for the management of anemia in dialysis patients will soon be released that may have a favorable impact on physician behavior and clinical management strategies. An example of such evidence-based practice

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guidelines are the National Kidney Foundation's Dialysis Outcomes Quality Initiative.

An improved characterization of the determinants of rhEPO-stimulated erythropoiesis in hemodialysis patients is essential. Such an analysis may permit the identification of novel areas for improved processes of anemia management. Pursuant to this task, a large, multicenter, nationally representative data base was used to examine the relationships between anemia and patient survival, and to identify the predictors of hemoglobin concentration.

Materials and Methods

National Medical Care, Inc. (Waltham, MA), a large provider of dialysis services in the United States, maintains a clinical data base that contains selected demographic, laboratory, and outcome information about end-stage renal disease (ESRD) patients receiving care in its dialysis facilities. These data are used to support a quality enhancement system called the Patient Statistical Profile system.

Patient Groups and Data

Patients receiving hemodialysis three times per week in National Medical Care dialysis units on January 1, 1993, were selected for this study. That sample included 21,899 patients. Laboratory files were searched for data acquired between October 1, 1992, and December 31, 1992. Patients with missing laboratory data were excluded from further analysis. The laboratory tests included hemoglobin, hematocrit, red blood cell count, white blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration, serum iron, serum ferritin, total iron-binding capacity (TIBC), transferrin saturation, serum albumin concentration, serum creatinine concentration, and URR. (URR is defined as the predialysis blood urea nitrogen [BUN] concentration minus the postdialysis BUN divided by the predialysis BUN multiplied by 100.) Other laboratory data included serum concentrations of sodium, potassium, chloride, calcium, lactate dehydrogenase, total bilirubin, total cholesterol, glucose, and uric acid. All tests were performed by a single clinical laboratory (LifeChem, Inc., Rockleigh, NJ). The average value of each test for the 3 mo of observation was calculated for each patient, and this mean quantity was used for subsequent analysis. Commonly measured laboratory variables were available for most patients, but TIBC and ferritin, for instance, were not available for all patients. Thus, the final sample with complete laboratory data was 14,896.

Age, sex, race, presence of diabetes mellitus, and baseline renal diagnosis for all participants were extracted from the Patient Statistical Profile data. Files were also searched for dose and frequency of rhEPO administration. No information was available in the data base on parenteral iron administration and on other comorbid conditions. In addition, no information was available on the patterns of supplemental folate administration. However, the standard of care is that most ESRD patients receive folate containing multivitamins.

Statistical Analyses

Simple descriptive statistics were performed for the major variables of interest. The association between hemoglobin and death was assessed first. The odds ratio (OR), *i.e.*, the odds of death among patients in a particular category of exposure divided by the corresponding odds in the comparison category, was used as the measure of association. OR were adjusted simultaneously for potentially confounding variables by multiple logistic regression analysis (7). The variables considered in these models were laboratory test results (compare with above) and patient-related variables (age, race, gender, diabetic status, and baseline renal diagnosis). The baseline renal diagnoses included glomerulonephritis, cystic disease, diabetic nephropathy, hypertension, myeloma, and renal failure of unknown origin, among others. If diabetes mellitus was reported but was not listed as the cause of renal failure, it was classified as a patient-related variable (comorbid condition). Patients with missing data were excluded; only those patients with complete data were included in these multivariable analyses (n = 14,896).

t test and ANOVA or corresponding nonparametric tests were used to evaluate the relationship between hemoglobin concentration and dichotomous and categorical variables. Pearson's correlation coefficient was used to evaluate the relationship between hemoglobin concentration and other continuous variables. Bivariate associations were evaluated using several transformations of raw data to determine the form of the association between variables or to normalize the distribution of variables, or both. Standard mathematical transformations were attempted (*e.g.*, log, square, square root, reciprocal, double reciprocal, multiplicative, exponential), and the association yielding the best statistical fit (*i.e.*, largest r^2 value) was chosen for presentation. Multiple linear regression was used to adjust simultaneously for potential confounding variables and to identify independent predictors of hemoglobin concentration (8). The variables of rhEPO administration (rhEPO dose and percentage of treatments that rhEPO was administered) were included first and fixed in the model. Laboratory and patient-related variables (age, race, gender, diabetic status, and baseline renal diagnosis) were then added to the model in a stepwise fashion. An automated stepwise function (proc general linear model) has been used to select the variables included in the model. No transformation or interaction among the various predictors has been examined. Only rhEPO-treated patients and patients with complete data were included in this analysis (n = 11,863).

All analyses were performed with the statistical package Statistical Analysis System (The SAS Institute, Cary, NC). Means are shown ± SD. All probability values are two-tailed.

Results

Patient and Erythrocyte Characteristics

Table 1 summarizes major characteristics and selected laboratory findings. Nearly half of the patients were women and 46.6% were white. Virtually all of the non-white patients were African-American. The median age of patients in 1993 was 62.1 yr. A clinical diagnosis of diabetes mellitus was made for 8497 patients (38.8%). The characteristics of the study group were similar to those of the total U.S. hemodialysis population except for racial distribution. The proportion of non-white patients (53.4%) was significantly larger in the present sample than in the general U.S. hemodialysis population (35%) (4). The mean hemoglobin was 95.2 g/L, and more than 90% of patients had hemoglobin and hematocrit values below the lower limit of normal for healthy individuals.

The analysis of red blood cell indices showed a normal MCV in 85% of patients (mean, 92.36 fl) but a low mean corpuscular hemoglobin concentration in 96% of the patients (mean, 308.7 g/L). Thus, an abnormal erythrocyte phenotype was evident in most patients. Mean iron levels were near the lower limit of normal, and TIBC levels were below the lower limit of normal. In contrast, the mean ferritin value of 288.7 ng/ml was elevated compared with normal values. The mean

Table 1. Descriptive characteristics of the study population[a]

Variable	Value	Reference Range[b] (units)
Age		
median	62.1	
mean (±SD)	59.7 (±14.8)	
Race (% white)	46.6	
Gender (% male)	49.1	
Diabetes (%)[c]	38.8	
Laboratory values (mean ± SD)		
hemoglobin	95.2 (±13.1)	120 to 170 (g/L)
hematocrit	30.9 (±4.1)	36.1 to 50.3 (%)
RBC	3.4 (±0.5)	4.2 to 6.1 ($\times 10^{12}$/L)
WBC	6.9 (±2.2)	4.8 to 10.8 ($\times 10^{12}$/L)
MCV	92.4 (±7.6)	80 to 99 (fl)
MCHC	308.7 (±12.8)	330 to 370 (g/L)
iron	10.3 (±5.05)	6.6 to 28.3 (μmol/L)
TIBC	43.9 (±9.6)	46.4 to 69.5 (μmol/L)
transferrin saturation	23.1 (±10.2)	20 to 50 (%)
ferritin	288.7 (±485.3)	5 to 179 (ng/ml)
albumin	37 (±4)	35 to 52 (g/L)
creatinine	981.2 (±318.2)	53 to 140 (μmol/L)
URR	62.9 (±8.4)	(%)

[a] n = 21,899. RBC, red blood cell; WBC, white blood cell; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; TIBC, total iron-binding capacity; URR, urea reduction ratio.

[b] Reference range from LifeChem, Inc. (Rockleigh, NJ).

[c] Present as the principal renal diagnosis or a comorbid condition.

albumin concentration for this population was 37 g/L (lower limit of normal), and the URR was 63%.

Eighty percent (80.6%) of patients received rhEPO, and ≥90% received it intravenously. The mean rhEPO dose was 3468 U/dose. Mean hemoglobin concentration among patients who received and who did not receive rhEPO was 95.7 ± 2.46 g/L and 95.1 ± 0.6 g/L, respectively (P = 0.015).

Association Between Hemoglobin and Death

Figure 1 shows the risk profile of death among hemoglobin categories adjusted for patient demographic variables (*i.e.*, age, gender, race, diabetic status, and renal diagnosis), for demographics and serum albumin, and for demographics, albumin, and other laboratory variables (labeled case mix, case mix + albumin, and case mix + laboratory-adjusted, respectively). The OR for death increased progressively among hemoglobin categories below 100 g/L. For example, hemoglobin concentrations ≤80 g/L were associated with a twofold increase in the odds of death (OR = 2.01; P = 0.001). Alternatively, hemoglobin concentrations exceeding 110 g/L were associated with no reduction in the OR for death. More than 10% (n = 2085) of the patients had hemoglobin concentrations >110 g/L, such that the lack of survival benefit was not likely because of a β-error. For hemoglobin concentrations <90 g/L, the OR for death were reduced when adjustment was made for serum albumin concentration. Adjustment for other laboratory variables did not change the results substantially (Figure 1).

Predictors of Hemoglobin Concentration

Table 2 summarizes the results from a multivariate, stepwise, linear regression analysis that evaluated the relationship between selected patient-related variables and laboratory tests

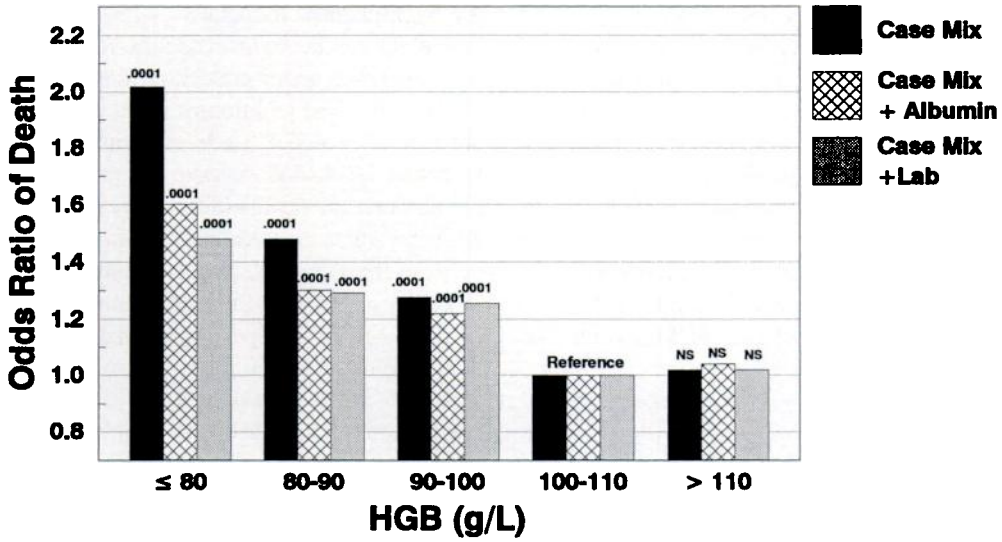

Figure 1. Risk of death according to hemoglobin concentration. The odds ratios are adjusted for patient characteristics only (age, gender, diabetic status, and renal diagnosis; black bars), for patient characteristics plus serum albumin concentration (hatched bars), and for patient characteristics, serum albumin concentration, and other routine laboratory variables (grey bars). P values are from comparison with the reference group. The number of patients in each hemoglobin category was: ≤80, n = 2266; 80 to 90, n = 4087; 90 to 100, n = 6071; 100 to 110, n = 4283; and >110, n = 2085.

Table 2. Multiple linear regression analysis with the hemoglobin concentration as the outcome variable[a]

Variable[b]	F^c	Regression Coefficient	SEM
Age	185	0.07	0.008
Race (ref = non-white)	144	2.66	0.221
Gender (ref = male)	117	−2.40	0.221
rhEPO dose	212	-6×10^{-4}	5×10^{-5}
% rhEPO[d]	931	−0.11	0.004
Iron	347	0.39	0.009
Transferrin saturation	43	0.10	0.002
TIBC	108	−0.11	0.002
Ferritin	122	-2.6×10^{-3}	0.003
Albumin	324	0.61	0.034
Creatinine	44	-3.1×10^{-3}	0.042
URR	40	0.08	0.012

[a] Includes only patients with rhEPO therapy and complete data (n = 11,863). All P values are <0.001. Overall r^2 = 0.27. ref, reference; rhEPO, recombinant human erythropoietin. Other abbreviations as in Table 1.

[b] Other significant, but less powerful, predictors of hemoglobin concentration examined in the analysis included anion gap and serum levels of glucose, cholesterol, calcium, bilirubin, LDH, and uric acid. Other variables not significantly associated with hemoglobin concentration included diabetic status, cause of renal failure, white blood cell count, and serum levels of sodium, potassium, and phosphorus. The variables rhEPO dose and % rhEPO were fixed before the addition of the other variables.

[c] F statistic in the final model. The greater the F value, the smaller the probability that chance alone is responsible for this finding.

[d] % rhEPO: percentage of treatments that rhEPO was administered.

with hemoglobin concentration in all patients receiving rhEPO (n = 11,863). The r^2 value for this linear model was 0.27, suggesting that 27% of the variability in hemoglobin concentration was accounted for by the variables included in the model. The partial r^2 values (a measure of the relative weight of influence) for race and age were 2.9 and 1.2%, respectively. Older age, white race, and male gender were individually associated with higher hemoglobin concentrations ($P < 0.001$). Each year of advancing age was associated with an increase in the mean hemoglobin concentration of 0.07 g/L. Whites had a mean hemoglobin concentration that was 2.66 g/L higher than non-whites, and women had a mean hemoglobin concentration that was 2.40 g/L lower than men.

Variables of rhEPO administration (rhEPO dose and percentage of treatments that rhEPO was administered) were inversely associated with the hemoglobin concentration ($P < 0.001$). Figure 2 illustrates the relationship between hemoglobin concentration and rhEPO dose among patients who received the drug with 90% or more of dialysis treatments. Higher doses of rhEPO were associated with lower hemoglobin concentrations ($r = -0.23$; $P < 0.001$).

Variables of iron status (serum iron, transferrin saturation, and ferritin) were also found to be significantly associated with hemoglobin concentrations ($P < 0.001$) (Table 2). Whereas both the serum iron concentration and transferrin saturation were directly associated with hemoglobin concentration ($P < 0.001$), the serum ferritin was found to be inversely correlated ($P < 0.001$). The partial r^2 value for ferritin was 0.4%. Figure 3 illustrates the bivariate associations between hemoglobin concentration and laboratory parameters of iron status. The association with serum iron concentration was fitted best with a curvilinear model (double reciprocal; $P < 0.001$). Higher serum iron values were associated with higher hemoglobin concentrations, with a plateau occurring for iron >60 mg/dl. A similar curvilinear relationship was also found for transferrin saturation, with a plateau in hemoglobin concentration occurring for transferrin saturations >20% (double reciprocal; $P < 0.001$). The association between hemoglobin and ferritin concentrations was inverse ($r = -0.007$; $P < 0.001$), fitting best into a linear model. These bivariate analyses do not permit further extrapolations.

Variables reflecting nutritional status (serum albumin and creatinine concentrations) were significantly associated with hemoglobin concentration (Table 2). Serum albumin concentration was highly and directly associated with hemoglobin ($P < 0.001$), but serum creatinine concentration was inversely correlated ($P < 0.001$). A change of 10 g/L in the serum albumin concentration was associated with a change of 6 g/L in the hemoglobin concentration. The partial r^2 value for albumin was 3.4%. The intensity of dialysis (*i.e.*, URR) was also found to be directly associated with the hemoglobin concentration ($P < 0.001$). Its partial r^2 value was 0.3%. Figure 4 shows the bivariate associations between hemoglobin concentration and albumin (Panel A) and URR (Panel B).

Other significant, but less powerful, predictors of hemoglobin concentration examined in the analysis included anion gap and serum concentrations of calcium, lactate dehydrogenase, bilirubin, glucose, cholesterol, and uric acid. Other variables not significantly associated with hemoglobin concentration included diabetic status, baseline renal diagnosis, white blood cell count, and serum concentrations of sodium, potassium, and phosphorus. The addition of other potential confounding variables in the model, such as reticulocyte count, parathyroid hormone level, and random serum aluminum concentration, did not alter the results of the regression model. Serum vitamin B12 and folate concentrations were not included in the model because these data were not available for most patients.

Because 19.4% of the study group did not receive rhEPO, a similar analysis was performed that included only patients not receiving rhEPO. The findings for these patients were consistent with those shown in Table 2. Serum iron and albumin concentrations were the first variables selected statistically by the modeling process. Similar associations between age, race, gender, ferritin concentration, URR, and other parameters were also found.

Discussion

Anemia has a significant impact on patient survival, although of lesser statistical magnitude than other predictors of death, such as serum albumin and creatinine concentrations

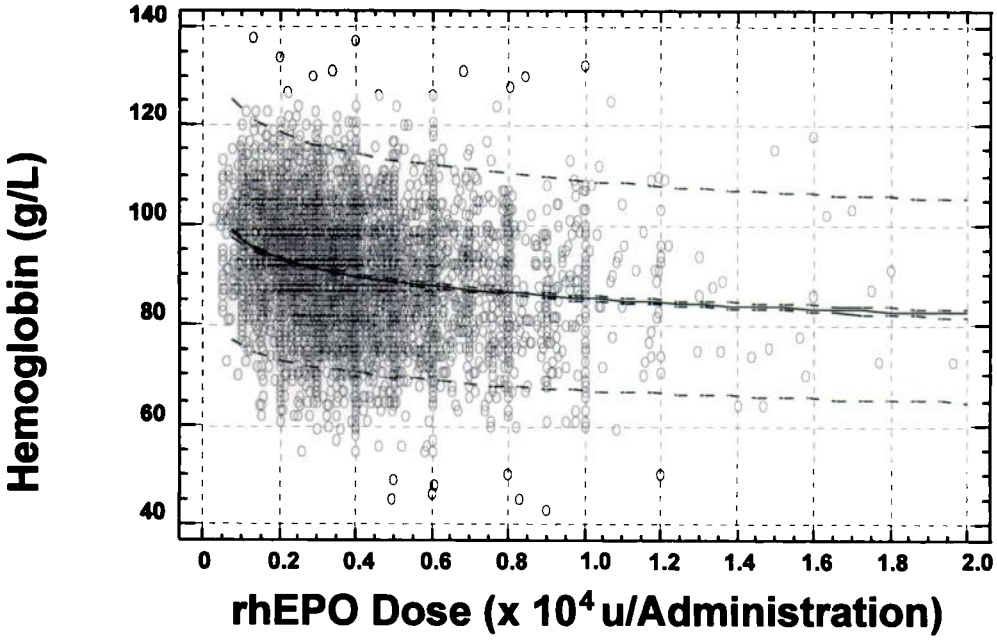

Figure 2. Relationship between hemoglobin concentration and dose of recombinant human erythropoietin (rhEPO) for patients who received rhEPO with >90% of their dialysis treatments ($r = -0.23$; $P < 0.001$). The regression line, 95% confidence limits, and 95% predictive limits are shown.

and the delivered dose of hemodialysis (2,3). In the present study, the relationship between hemoglobin concentration and survival was examined further to define an optimal concentration based on the OR for death. It was observed that after adjustment for other demographic and laboratory predictors of survival, patients with a hemoglobin concentration <80 g/L had an approximate 50% increase in the odds of death compared with patients with a hemoglobin concentration of 100 to 110 g/L. Furthermore, hemoglobin concentrations exceeding 110 g/L were not associated with further reduction in the odds of death.

Because of the inability to gather more extensive information about patient comorbidity, and to incorporate this into the statistical constructs, these data demonstrate that dialysis patients corrected to a hemoglobin of 100 to 110 g/L had the lowest mortality. It is possible that patients with the worst anemia had the greatest comorbid conditions, and the increased death risk was a result of this confounding effect. This distinction is critical in clarifying the role of anemia correction to reduce death risk. The data presented here do not permit any greater distinction in data interpretation.

Previous studies have attempted to define the optimal concentration to which hemoglobin should be corrected during rhEPO therapy in terms of patient morbidity, quality of life, and exercise capacity (9–12). Intervention trials with rhEPO have suggested that most of the quality-of-life benefit from rhEPO's correction of anemia was achieved at a hemoglobin concentration of approximately 105 g/L (9). However, recent studies have suggested that not only did quality of life benefits extend beyond this cutoff, but other benefits, such as left ventricular hypertrophy and improvement in heart function, may accrue when higher concentrations of hemoglobin were reached (10–12). Recent studies have also related the hemoglobin concentration to the development of congestive heart failure (13), and all-cause death rates have been shown to be lower in patients with a mean hematocrit of 32% than in patients with a mean hematocrit of 26% (14). Importantly, none of the previous studies has attempted to define the optimal hemoglobin concentration in terms of patient mortality. The current study suggests that for many patients, hemoglobin levels >110 g/L may not be associated with a significant benefit in terms of overall patient mortality. Of note, a recent randomized control trial evaluating the impact of two target hematocrits (42 and 30%) on the mortality of hemodialysis patients with cardiac disease was stopped prematurely based on recommendations of the external monitoring board. A trend toward greater patient mortality in the high hematocrit group (Hct = 42%) compared with the low hematocrit group (Hct = 30%) was observed (A. Nissenson, J. Eschbach, personal communication).

To eventually optimize the hemoglobin concentration, independent predictors of hemoglobin concentration must be identified. Age, race, and gender were found to be significant demographic variables associated with hemoglobin concentration. Male patients and white patients were found to have higher hemoglobin values when adjusting for rhEPO dose, iron and ferritin levels, and albumin concentration. Similar differences are found in the general population and are not specific to ESRD patients on hemodialysis (15,16).

Variables of rhEPO administration (rhEPO dose and percentage of treatments that rhEPO was administered) were also identified as independent predictors of hemoglobin concentra-

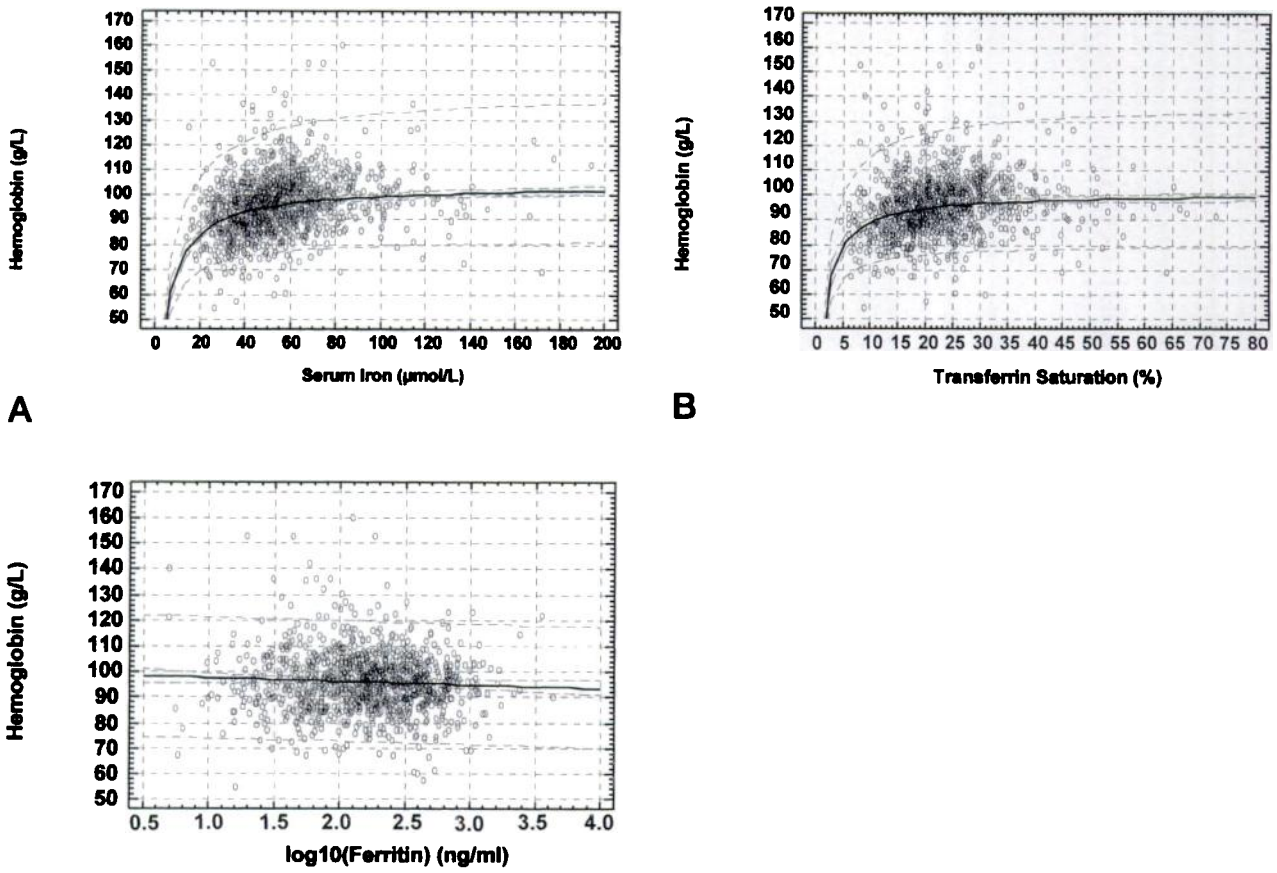

Figure 3. Bivariate associations between hemoglobin concentration and serum iron concentration (A), transferrin saturation (B), and serum ferritin concentration (log-transformed; C). The regression line, 95% confidence limits, and 95% predictive limits are shown and were constructed using all of the data. Only a 5% random sample of the data points is shown here.

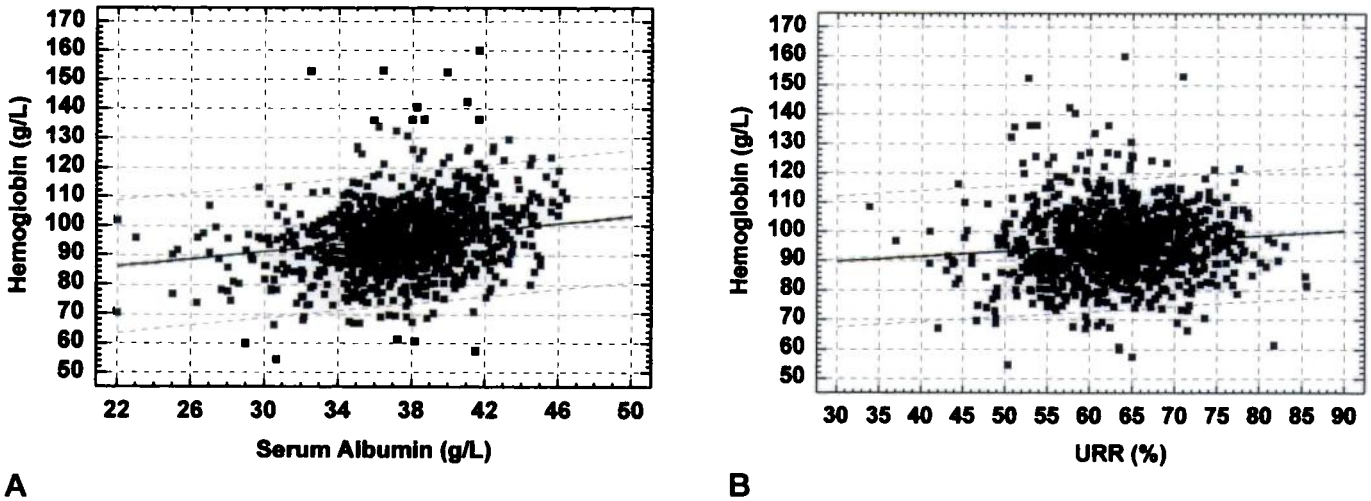

Figure 4. Bivariate associations between hemoglobin concentration and serum albumin concentration (A) and urea reduction ratio (B) ($P < 0.001$). The regression line, 95% confidence limits, and 95% predictive limits are shown and were constructed using all of the data. Only a 5% random sample of the data points is shown here.

tion. Both were inversely associated with the hemoglobin concentration. This counter-intuitive finding may reflect physician practice or differential rhEPO responsiveness, or both. rhEPO is prescribed to improve hemoglobin or hematocrit to approach the threshold value at which reimbursement from the Health Care Financing Administration ceases. As that point

nears, the dose of rhEPO is decreased. Alternatively, if the hemoglobin fails to improve, the dose of rhEPO is increased. A similar phenomenon occurs with blood transfusions; severely anemic patients receive more transfusions than mildly anemic ones.

The independent positive association of serum iron level and transferrin saturation with hemoglobin concentration is easily understood and underscores the critical role of iron in achieving optimal response to rhEPO therapy. The transferrin saturation corresponds to iron that is readily available for erythropoiesis and is one of the most widely used and reliable indicators of iron status (17,18). The curvilinear relationship between transferrin saturation and hemoglobin concentration indicates that the hemoglobin concentration reaches a plateau of 100 to 110 g/L at transferrin saturation ≥20%. Thus, whereas some patients may individually benefit from a higher transferrin saturation, the population as a whole does not appear to benefit in terms of hemoglobin concentration. With regard to patient management, until a prospective analysis is performed, iron replacement should be aimed at maintaining a transferrin saturation of at least 20%. Further study is needed to evaluate whether patient subsets exist that may benefit from a higher transferrin saturation.

The finding of ferritin as an independent inverse predictor of hemoglobin concentration may have several explanations. First, high ferritin levels, observed in association with low normal serum iron, TIBC, and transferrin saturation, are commonly found in anemic patients with chronic diseases and suggest impaired iron metabolism (19–21). Second, these data suggest that ferritin concentration in dialysis patients is not a simple measure of iron stores. Because ferritin synthesis is enhanced during inflammation, high ferritin levels may be a consequence of a nonspecific systemic inflammatory process rather than of increased iron stores. In this circumstance, the inflammatory process that stimulates ferritin synthesis may also act to inhibit erythropoiesis. Bone marrow hypoproliferation may occur because of endogenous inhibitors, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6. Nude mice injected with transfected cells with TNF-α develop a marked hypoproliferative anemia associated with a reduction in erythroid colony-forming units and burst-forming units (22). In the absence of infection, autoimmune disorders, and neoplastic diseases, elevated levels of IL-1 and TNF-α have been observed in dialysis patients (23). Furthermore, elevated levels of IL-6 have been shown to be associated with impaired erythropoiesis and resistance to rhEPO in dialysis patients (24). Therefore, the increased serum ferritin concentration may be a surrogate of cytokine-dependent inhibition of erythropoietin.

In addition, inflammation is also associated with a defect in iron utilization, characterized by reduced iron delivery (25). In the face of inflammatory blockade, iron stored within reticuloendothelial cells is not released to transferrin. However, the transferrin-to-erythroid progenitor cell transfer remains intact. Serum iron and transferrin saturation are typically reduced, and marrow response to erythropoietin is severely limited by this relative iron deficiency (25).

Of all the variables examined in this study, albumin was the most closely associated with hemoglobin concentration; hence, higher serum albumin concentrations were associated with greater hemoglobin concentrations. This finding was also observed by others, without comment (26). If albumin is viewed as a surrogate of visceral protein nutrition, then hypoalbuminemia is a marker for visceral protein depletion, which may contribute to impaired erythropoiesis and anemia. Visceral protein deficiency could adversely affect heme and erythrocyte synthesis, as well as iron transport, storage, and utilization (27). It could also affect erythropoietin production, although this effect is minimal in the anephric state of ESRD. The importance of dietary protein in the synthesis of hemoglobin has been reported in both animal and clinical human studies (28–30). Anemia is a common feature of patients with kwashiorkor secondary to low protein and calorie intake (27,31–34). A direct correlation between serum protein levels and hemoglobin concentration has been observed (32,34). Moreover, several investigators have described complete correction of anemia in patients with normal renal function and protein energy malnutrition after treatment with high-protein diets alone (31,35,36).

It is noteworthy that the serum albumin concentration directly correlated with erythropoiesis, but that the serum creatinine concentration correlated inversely. If the nutritional status alone were the principal determinant, both of these laboratory surrogates of nutrition should be directly predictive of erythropoietic response. Thus, in some patients, the reduction in albumin concentration may not be a surrogate of low-protein calorie intake, but a consequence of decreased albumin synthesis independent of nutritional status (37). Hypoalbuminemia without malnutrition has been observed in hemodialysis patients and has been ascribed to the effect of inflammation on hepatic albumin synthesis (37). If this mechanism is operational, hypoalbuminemia and anemia are linked as concurrent non-nutritional occurrences. Inflammation results in both decreased albumin synthesis and anemia. Support for this hypothesis is provided by the observed inverse correlation between ferritin levels and hemoglobin concentration.

Hemoglobin concentration was also found to be significantly correlated with URR, a measure of dialysis intensity. Similar results were reported in which the investigators observed a positive correlation between hemoglobin concentration and URR (26). An increase in URR was associated with an increment in the hematocrit value.

Another novel finding was that the majority of patients had a severely hypochromic normocytic anemia. These erythrocytes are different from the microcytic, hypochromic cells of iron deficiency and the classical normocytic normochromic cells that are seen in patients with chronic diseases but normal renal function. Similarly, this erythrocyte phenotype is not observed in chronic renal failure without erythropoietin therapy (38). The precise reasons for this finding are unclear. However, a kinetic imbalance between rhEPO and iron delivery to the bone marrow, rather than an absolute iron deficit, could explain the finding of erythrocytes of normal size (normal MCV) with low hemoglobin content (low hemoglobin

concentration). Because high levels of rhEPO are observed immediately after intravenous injections of the hormone, normoblasts undergo rapid proliferation and hemoglobin synthesis. The resultant hematopoietic demand for iron is so tremendous that hemoglobin synthesis by normoblasts exceeds iron availability, despite normal or low-normal iron stores, and relatively poorly hemoglobinized reticulocytes and erythrocytes appear in circulation (39–42). However, these erythrocytes may lack the microcytosis that is the hallmark of absolute iron deficiency (43), because rhEPO forces the premature differentiation and release of less mature, and hence larger, cells in the erythroid lineage. Thus, although iron-deficient and hypochromic, these erythrocytes may not be microcytic (44). By coordinating the administration of rhEPO and iron by subcutaneous dosing of the former in parallel with routine maintenance intravenous administration of the latter, the kinetic imbalance between rhEPO and iron delivery to the bone marrow might be minimized.

The cause of anemia in dialysis patients is complex and multifactorial. Although the predominant reason for the development of anemia is the lack of erythropoietin production by the diseased kidney, this study identified other important contributing factors that must be taken into consideration. These factors, such as protein energy malnutrition, iron deficiency, and delivered dose of hemodialysis, merit renewed attention because of their potential significant impact on anemia, rhEPO responsiveness, and ultimately patient survival. Correction of anemia through interventions targeting these factors may provide improved survival for patients on maintenance hemodialysis. Prospective, interventional studies will be required to validate this hypothesis.

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References

1. Owen W, Lew N, Liu Y, Lowrie E, Lazarus J: The urea reduction ratio and serum albumin concentration as predictors of mortality in patients undergoing hemodialysis. *N Engl J Med* 329: 1001–1006, 1993
2. Lowrie E, Lew N: Death risk in hemodialysis patients: The predictive value of commonly measured variables and an evaluation of death rate differences between facilities. *Am J Kidney Dis* 15: 458–482, 1990
3. Lowrie E, Lew N: Commonly measured laboratory variables in hemodialysis patients: Relationships among them and to death risk. *Semin Nephrol* 12: 276–283, 1992
4. Held P, Port F, Webb R, Wolfe RA, Bloembergen WE, Turenne MN, Holzmann E, Ojo AO, Young EW, Mauger EA: Excerpts from United States Renal Data System 1995 Annual Report. *Am J Kidney Dis* 26[Suppl 2]: S1–S186, 1995
5. Paganini E, Eschbach J, Lazarus J, van Stone JC, Gimenez LF, Graben SE, Egrie JC, Okamoto DM, Goodkin DA: Intravenous versus subcutaneous dosing of Epoetin Alfa in hemodialysis patients. *Am J Kidney Dis* 26: 331–340, 1995
6. Fishbane S, Frei G, Maesaka J: Reduction in recombinant human erythropoietin doses by the use of chronic intravenous iron supplementation. *Am J Kidney Dis* 26: 41–46, 1995
7. Hosmer D, Lemeshow S: *Applied Logistic Regression*, New York, John Wiley & Sons, 1989
8. Kleinbaum D, Kupper L, Muller K: *Applied Regression Analysis and other Multivariate Methods*, Boston, PWS KENT Publishing, 1988
9. Canadian Erythropoietin Study Group: Association between recombinant erythropoietin and quality of life and exercise capacity of patients receiving haemodialysis. *Br Med J* 300: 573–578, 1990
10. Eschbach J, Glenny R, Robertson T: Normalizing the hematocrit in hemodialysis patients with rhEPO improves quality of life and is safe [Abstract]. *J Am Soc Nephrol* 4: 425, 1993
11. Low-Friedrich I, Grutzmacher P, Marz W, Bergmann M, Schoeppe W: Therapy with recombinant erythropoietin reduces cardiac size and improves cardiac function in chronic hemodialysis patients. *Am J Nephrol* 11: 54–60, 1991
12. MacDougall I, Lewis N, Saunders M: Long-term cardiorespiratory effects of amelioration of renal anemia by erythropoietin. *Lancet* 335: 489–493, 1990
13. Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC, Barre PE: The impact of anemia on cardiomyopathy, morbidity, and mortality in end-stage renal disease. *Am J Kidney Dis* 28: 53–61, 1996
14. Collins A, Ma J, Umen A: Infectious and all-cause death rates are lower with a mean hematocrit of 32% versus 26% [Abstract]. Proceedings of the International Society of Nephrology Meeting, Jerusalem, 1993, p 386
15. Perry G, Byers T, Yip R, Margen S: Iron nutrition does not account for the hemoglobin differences between blacks and whites. *J Nutr* 122: 1417–1424, 1992
16. Meyers L, Habicht J, Johnson C: Components of the differences in hemoglobin concentration in blood between black and white women in the United States. *Am J Epidemiol* 109: 539–549, 1979
17. VanWyck D, Stivelman J, Ruiz J, Kirlin L, Katz M, Ogden D: Iron status in patients receiving erythropoietin for dialysis-associated anemia. *Kidney Int* 35: 712–716, 1989
18. Muirhead N, Bargman J, Burgess E, Jindal KK, Levin A, Nolin L, Parfrey P: Evidence-based recommendations for the clinical use of recombinant human erythropoietin. *Am J Kidney Dis* 26: S1–S24, 1995
19. Baynes R, Flax H, Bothwell T: Hematologic and iron related measurements in active pulmonary tuberculosis. *Scand J Hematol* 36: 280–287, 1986
20. Cash J, Sears D: The anemia of chronic disease: Spectrum of associated diseases in a series of unselected hospitalized patients. *Am J Med* 87: 638–644, 1989
21. Means R, Drantz S: Progress in understanding the pathogenesis of the anemia of chronic disease. *Blood* 80: 1639–1647, 1992
22. Roodman G, Johnson G, Clibon U: Tumor necrosis factor-alpha and the anemia of chronic disease: Effects of chronic exposure to TNF on erythropoiesis in vivo. *Adv Exp Med Biol* 271: 185–196, 1989
23. Pereira J, Shapiro L, King A, Falagas M, Strom J, Dinarello C: Plasma levels of IL-1β, TNF-α and their specific inhibitors in undialyzed chronic renal failure, CAPD, and hemodialysis patients. *Kidney Int* 45: 890–896, 1994
24. MacDougall I, Allen D, Tucker B, Baker L, Baker A: Serum interleukin-6 levels are a useful indicator of marrow suppression

in patients with resistance to erythropoietin due to inflammatory disease [Abstract]. *J Am Soc Nephrol* 4: 428, 1993
25. Kleiner M, VanWyck D, Kauple C, Kirlin L: The role of iron and other factors in patients unresponsive to erythropoietin therapy. *Semin Dialysis* 8: 29–34, 1995
26. Ifudu O, Feldman J, Friedman EA: The intensity of dialysis and the response to erythropoietin in patients with end-stage renal disease. *N Engl J Med* 334: 420–425, 1996
27. Edozien J, Rahim-Khan M: Anemia in protein malnutrition. *Clin Sci (Lond)* 34: 315–326, 1968
28. Ramalingaswami V, Deo M, Sood S: *Protein Deficiency in the Rhesus Monkeys,* Washington, DC, National Academy of Science, National Research Council, Publication no. 843, 1961, p 365
29. Whipple G, Hooper C: Blood regeneration after simple anemia: Curve of regeneration influenced by dietary factors. *Am J Physiol* 45: 573–577, 1918
30. Jencks Z: Studies in the regeneration of blood. *Am J Physiol* 59: 240–253, 1922
31. Abdel-Salam E, Allah AK, Osman N: Anemia of protein malnutrition. *Acta Biol Med Germ* 27: 279–288, 1971
32. Keys A, Brozeck J, Henschel A, Elsen OM, Taylor H: *The Biology of Human Starvation,* Minneapolis, University of Minnesota Press, 1950, p 1385
33. MacDougall L, Moodley G, Eyberg C, Quirk M: Mechanisms of anemia in protein-energy malnutrition in Johannesburg. *Am J Clin Nutr* 35: 229–235, 1982
34. Leyton G: Effects of slow starvation. *Lancet* 2: 73–79, 1946
35. Woodruff A: *Recent Advances in Human Nutrition,* edited by Brock JF, London, Churchill, 1961
36. Olsen R: The anemia of protein-calorie malnutrition and its response to dietary protein. *Nutr Rev* 37: 81–83, 1979
37. Kaysen G, Rathore V, Shearer G, Depner T: Mechanisms of hypoalbuminemia in hemodialysis patients. *Kidney Int* 48: 510–516, 1995
38. Bunn H: Anemia associated with chronic disorders. In: *Principles of Internal Medicine,* edited by Wilson JD, Braunwald E, New York, McGraw-Hill, 1991, pp 1529–1531
39. Brugnara C, Colella G, Cremins J: Effects of subcutaneous recombinant human erythropoietin in normal subjects: Development of decreased hemoglobin content and iron-deficiency erythropoiesis. *J Lab Clin Med* 123: 660–667, 1994
40. MacDougall I, Cavill I, Hulme B: Detection of functional iron deficiency during erythropoietin treatment: A new approach. *Br Med J* 304: 225–226, 1992
41. Goodnough L, Price T, Rudnick S: Iron-restricted erythropoiesis as a limitation to autologous blood donation in the erythroid-stimulated bone marrow. *J Lab Clin Med* 118: 289–296, 1991
42. Brugnara C, Laufer M, Friedman A, Bridges K, Platt O: Reticulocyte hemoglobin content: Early indicator of iron deficiency and response to therapy. *Blood* 83: 3100–3101, 1994
43. Lin C, Lin J, Chen S, Jiang M, Chiu C: Comparison of hemoglobin and red blood cell distribution width in the differential diagnosis of microcytic anemia. *Arch Pathol Lab Med* 116: 1030–1032, 1992
44. D'Onofrio G, Chirillo R, Zini G, Caenaro G, Tommasi M, Micciulli G: Simultaneous measurement of reticulocytes and red blood cell indices in healthy subjects and patients with microcytic and macrocytic anemia. *Blood* 83: 818–823, 1995