Kidney International, Vol. 66 (2004), pp. 1131–1138

Anemia and end-stage renal disease in patients with type 2 diabetes and nephropathy

ANUPAMA MOHANRAM, ZHONGXIN ZHANG, SHAHNAZ SHAHINFAR, WILLIAM F. KEANE, BARRY M. BRENNER, and ROBERT D. TOTO

UT Southwestern Medical Center, Dallas, Texas; Merck & Co., West Point, Pennsylvania; and Brigham and Women's Hospital, Boston, Massachusetts

Anemia and end-stage renal disease in patients with type 2 diabetes and nephropathy.

Background. Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD). Anemia is common in diabetics with nephropathy; however, the impact of anemia on progression to ESRD has not been carefully examined.

Methods. We studied the relationship between baseline hemoglobin concentration (Hb) and progression of diabetic nephropathy to ESRD in 1513 participants enrolled in Reduction in Endpoints in NIDDM with the Angiotensin II Antagonist Losartan study and followed for an average of 3.4 years. Multivariate Cox proportional hazards models were used to analyze the relationship between Hb and ESRD, after adjusting for predictors for ESRD. Analyses were performed with Hb stratified by quartile: first quartile <11.3 g/dL, second quartile 11.3 to 12.5 g/dL, third quartile 12.6 to 13.8 g/dL, and fourth quartile ≥13.8 g/dL (reference) and as a continuous variable.

Results. Baseline hemoglobin concentration was correlated with subsequent development of ESRD. After adjustment for predictors of ESRD, the hazard ratios for the first, second, and third Hb quartiles were 1.99 (95% CI, 1.34-2.95), 1.61 (95% CI 1.08-2.41), and 1.87 (95% CI 1.25-2.80). With hemoglobin as a continuous variable, the adjusted hazard ratio was 0.90 (95% CI 0.84-0.96, $P = 0.0013$). The average increase in adjusted relative risk was 11% for each 1 g/dL decrease in hemoglobin concentration.

Conclusion. Our data suggest that even mild anemia, Hb <13.8 g/dL increases risk for progression to ESRD. Hemoglobin is an independent risk factor for progression of nephropathy to ESRD in type 2 diabetes.

Diabetic nephropathy is a major public health problem in the United States. It is the leading cause of end-stage renal disease (ESRD), accounting for nearly 50% of all new cases at a cost of $6 billion per year, consuming approximately 3% of the Medicare budget [1]. Risk factors for development and progression of diabetic kidney disease include hypertension, poor glycemic control, and levels of proteinuria. Lowering blood pressure and using angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists slow progression of diabetic nephropathy; however, these treatments do not stop progression of disease to end-stage. One possibility for continued progression of kidney disease is the presence of unrecognized risk factors.

Anemia is common among patients with nephropathy attributed to type 2 diabetes and is a known risk factor for morbidity and mortality among patients with ESRD [2–7]. Several small studies have suggested that low hemoglobin concentration is a risk factor for progression of both diabetic and nondiabetic nephropathies. However, these studies were limited by retrospective design, small numbers of enrollees, surrogate markers of renal outcome, and short duration of follow-up [8–17]. In a preliminary analysis of the Reduction in Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study, hemoglobin at baseline was identified as an independent predictor for progression of nephropathy in participants with type 2 diabetes [18]. Because there are therapeutic options for treatment of anemia, we sought to evaluate anemia further as a risk factor for ESRD in nephropathy attributed to type 2 diabetes.

METHODS

Study population

The RENAAL study was a prospective randomized, multinational, double-blind, placebo-controlled trial of 1513 participants with type 2 diabetes and nephropathy who were followed for an average of 3.4 years. The primary end point of RENAAL was the combined end point of doubling of serum creatinine, ESRD, or death. The study protocol was approved by the institutional review board at each center, and all patients provided written informed consent. The inclusion and exclusion criteria and description of the RENAAL population have been

Key words: anemia, ESRD, progression, multivariate analysis, diabetic nephropathy.

Received for publication October 7, 2003
and in revised form February 12, 2004, and March 25, 2004
Accepted for publication April 5, 2004

reported. The RENAAL study demonstrated a renoprotective effect of losartan (Cozaar; Merck & Co., West Point, Pa, USA) in type 2 diabetics with nephropathy, beyond blood pressure control [19, 20].

Hematology/blood sampling

All baseline laboratory parameters in RENAAL were determined prior to randomization [19]. Hemoglobin concentration was chosen because it is the standard indicator for anemia among those with chronic kidney disease (CKD) [21]. Baseline hemoglobin concentration was determined for each patient from a single measurement. Samples for hemoglobin analysis were collected in potassium EDTA tubes at room temperature and analyzed in regional reference laboratories. Hemoglobin measurement was determined by photometric absorbance using Coulter model GEN-S and STKS (Beckman Coulter, Inc., Fullerton, CA, USA). Hemoglobin measurements were cross-validated on a monthly basis using normal and abnormal samples in the central laboratory.

End points

The principal end point for this analysis was ESRD defined as the need for chronic dialysis or renal transplantation. We also evaluated the composite end point of ESRD or death because death is an important competing event for patients with CKD [22, 23]. Death was defined as death from any cause as prespecified in the RENAAL study protocol [19]. For our analysis, if the participant reached ESRD and died later in the study, ESRD was counted as the end point.

Ascertainment of end points

One hundred percent of the participants could be accounted for with respect to the end points of ESRD and death. Households of participants who did not return for follow-up visits were contacted by telephone to determine if the participants had started dialysis, undergone renal transplant, or died. In some cases, further information was obtained from the Centers for Medicare and Medicaid Services for the ESRD end point, and from the Social Security Administration Death Index for the death end point. All study end points were adjudicated by an independent Clinical Endpoint Classification Committee blinded to the treatment arm of the study participant, in accordance with study protocol [19].

Statistical analysis

In order to explore the relationship between hemoglobin and end points, participants were categorized by quartiles according to baseline hemoglobin concentration (Hb): first quartile <11.3 g/dL, second quartile 11.3 to 12.5 g/dL, third quartile 12.6 to 13.8 g/dL, and fourth quartile ≥13.8 g/dL. The fourth quartile was designated as the reference quartile to which all other quartiles were compared.

The baseline characteristics for participants were summarized by Hb quartile with mean and standard deviation for continuous variables and number and percent for categorical variables. Trend tests were done between Hb quartiles (independent variable) and each covariate (dependent variable) using linear regression for continuous variables, and the Cochran-Armitage test for trend for categorical variables [24]. Each categorical variable was dichotomized into a binary variable. For example, for race, four binary variables were created: Asian versus non-Asian, black versus non-black, Hispanic versus non-Hispanic, and white versus non-white.

Event curves depicting the percentage of patients experiencing both ESRD and ESRD or death were estimated by Hb quartile using the Kaplan-Meier procedure. The risk for each end point was summarized by Hb quartile and expressed as event rate in units of events per 1000 patient-years of follow-up. The event rate per 1000 patient-years of follow-up was calculated as $1000 \times$ (total number of patients with event)/(total number of years until event or study termination). In addition, the relative hazard risk (expressed as the hazard ratio) for Hb for each end point was evaluated using Cox proportional hazards models with Hb as a categorical variable (quartiles) and as a continuous variable.

End-stage renal disease end point

Multivariate Cox regression models were utilized to identify hemoglobin as an independent risk factor and to explore the relationship between hemoglobin and ESRD. Unadjusted Cox regression models were utilized to calculate hazard ratios with respect to the highest Hb quartile. Additional covariates for the primary multivariate model were selected a priori based on scientific evidence demonstrating influence on progression of kidney disease as follows: age, sex, race, body mass index, smoking, systolic blood pressure (SBP), diastolic blood pressure (DBP), serum creatinine, serum albumin, calcium, phosphorus, total cholesterol, low-density lipoprotein, triglycerides (natural log transformed), and proteinuria (natural log transformed). In this model, categorical covariates were converted into binary indicators: male (=1) versus female (=0), Asian (=1) versus non-Asian (=0) and so on. Covariates from this list that were significant on univariate analysis were entered into a multivariate model. Backward selection was used to determine the significant covariates with $\alpha = 0.01$. Through this sequence, four independent covariates were identified: Hb (either as a continuous variable or by quartiles), serum creatinine, proteinuria, and serum albumin. Adjusted hazard ratios were calculated with respect to the highest Hb

quartile from a final multivariate model that included Hb quartiles, proteinuria, serum creatinine, and serum albumin, the independent risk factors identified through this process.

To explore our model further, we developed additional multivariate models with the following covariates added to the above list of baseline covariates: estimated glomerular filtration rate [21], baseline medical history (amputation, angina, congestive heart failure, myocardial infarction, neuropathy, and retinopathy), and randomization to losartan. In the initial and additional multivariate models for the end point of ESRD, the same four independent covariates were identified: Hb, serum creatinine (or estimated glomerular filtration rate), proteinuria, and serum albumin. Randomization to losartan was also an independent covariate when included in the model, but did not change our conclusion.

End-stage renal disease or death end point

The same procedure was used to develop a separate initial multivariate model for the end point of ESRD or death, utilizing the a priori list of baseline covariates as described for ESRD. Additional multivariate analyses for ESRD or death were repeated with the following sets of additional covariates: estimated glomerular filtration rate, baseline medical history, and randomization to losartan. For the ESRD or death end point, the initial and additional models revealed the following independent risk factors: Hb, serum creatinine (or estimated glomerular filtration rate), proteinuria, and serum albumin. Hazard ratios were calculated for the end point ESRD or death from the final multivariate model with Hb quartiles, proteinuria, serum creatinine, and serum albumin, the independent risk factors identified through this process. Randomization to losartan was also an independent covariate when included in the model, but did not change our conclusion.

Because Hb was not available for 45 of these participants, it was predicted through linear regression analysis modeled on Hb, with available baseline covariates for all participants. Other baseline covariates were not available for 15 participants. Analyses excluding these 60 patients (4%) were also conducted. Participants with missing data at baseline were not randomly distributed across Hb quartiles. Most of the participants with missing data at baseline were in the third Hb quartile (46.7%), while few were in the fourth Hb quartile (8.3%). In order to circumvent the nonrandom nature of missing data at baseline, imputation was used to predict missing values as described in order to include all participants in the analysis. The above models were repeated while excluding 60 patients with missing values at baseline, and similar results were obtained for both end points. Statistical Analysis System Version 8 (Cary, NC, USA) was used for all analyses.

RESULTS

Baseline characteristics

Participant characteristics by Hb quartile are shown in Table 1. Overall, participants were older [60 ± 7 years old (mean $\pm$ SD)], mostly male (63%), had elevated serum creatinine (1.9 ± 0.5 mg/dL), and had significant proteinuria measured as urine albumin/creatinine ratio (1810.6 ± 1735.4 mg/g). On average, participants had hypoalbuminemia, hypercholesterolemia, and hypocalcemia, and were moderately overweight. Average SBP was 153 ± 19 mm Hg, and average DBP was 82 ± 10 mm Hg. Eighteen participants were receiving erythropoietin. On trend analysis ($P < 0.05$), female gender, African American race, neuropathy, retinopathy, increasing SBP, phosphorus, proteinuria, and LDL were associated with lower Hb. White race, smoking, myocardial infarction history, increasing DBP, albumin, and calcium were associated with higher Hb.

Hemoglobin distribution

Baseline hemoglobin for this study population is illustrated in Figure 1. Hb ranged from 6.8 g/dL to 18.0 g/dL, with a mean and standard deviation of 12.5 ± 1.8 g/dL. Median hemoglobin was 12.4 g/dL. Randomization to losartan treatment was balanced in each quartile (49.2% for the first quartile, 50.1% for the second quartile, 47.4% for the third quartile, 51.6% for the fourth quartile). At baseline, the mean hemoglobin concentration was 12.5 g/dL in both the placebo group and the losartan group. After 36 months, the average follow-up hemoglobin concentration was lower in both the losartan group (11.9 g/dL) and the placebo group (12.3 g/dL).

End-stage renal disease

The proportion of participants reaching ESRD over time was significantly higher for those in the first, second, and third Hb quartiles compared to those in the fourth quartile (Fig. 2). Participants with lower Hb had higher event rates for ESRD (Table 2). The event rates for ESRD per 1000 patient-years of follow-up were 144.2 for the lowest quartile, 84.5 for the second quartile, 75.6 for the third quartile, and 27.6 for the fourth quartile. The unadjusted hazard ratios were 5.87 (95% CI 4.03-8.55, $P < 0.001$) for the lowest quartile, 3.25 (95% CI 2.19-4.82, $P < 0.001$) for the second quartile, and 2.86 (95% CI 1.91-4.28, $P < 0.001$) for the third quartile, compared to the fourth quartile. After adjustment for other baseline risk factors, the risk for ESRD in the first, second, and third Hb quartiles was still significantly greater compared to the fourth quartile (Table 2).

Addition of baseline estimated glomerular filtration rate and components of the medical history, along with prespecified covariates (see **Methods**) in the multivariate model did not change the results. The multivariate model

Table 1. Baseline clinical characteristics by quartile of hemoglobin concentration[a]

	Baseline hemoglobin quartile				*P* value for trend[c]
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
	Hb <11.3 g/dL	11.3–12.5	12.5–13.8	Hb ≥13.8 g/dL	
Baseline characteristic	*N* = 372	*N* = 368	*N* = 337	*N* = 391	
Age *years*	59.0 ± 7.6	60.4 ± 7.6	60.9 ± 6.8	60.3 ± 7.6	0.014
Sex					
Male no. (%)	144 (38.7)	214 (58.2)	236 (70.0)	331 (84.7)	<0.001
Female no. (%)	228 (61.3)	154 (41.8)	101 (30.0)	60 (15.3)	<0.001
Race[b]					
Asian no. (%)	84 (22.6)	52 (14.1)	44 (13.1)	59 (15.1)	0.0006
Black no. (%)	74 (19.9)	75 (20.4)	38 (11.3)	39 (10.0)	<0.001
Hispanic no. (%)	78 (21.0)	69 (18.8)	66 (19.6)	60 (15.3)	0.063
White no. (%)	133 (35.8)	164 (44.6)	185 (54.9)	229 (58.6)	<0.001
Sitting systolic BP *mm Hg*	155.0 ± 19.9	153.2 ± 19.2	152.5 ± 19.8	149.6 ± 18.4	<0.001
Sitting diastolic BP *mm Hg*	80.4 ± 10.6	81.5 ± 10.0	82.4 ± 10.7	84.8 ± 9.8	<0.001
Serum creatinine *mg/dL*	2.1 ± 0.5	1.9 ± 0.5	1.8 ± 0.5	1.7 ± 0.4	<0.001
Estimated GFR *mL/min/1.73m²*	33.3 ± 10.8	38.9 ± 12.0	41.0 ± 11.8	45.9 ± 11.5	<0.001
Serum albumin *g/dL*	3.6 ± 0.4	3.7 ± 0.4	3.8 ± 0.4	4.0 ± 0.4	<0.001
Calcium *mg/dL*	9.1 ± 0.5	9.3 ± 0.5	9.4 ± 0.5	9.5 ± 0.4	<0.001
Phosphorus *mg/dL*	4.2 ± 0.6	4.0 ± 0.6	3.8 ± 0.6	3.6 ± 0.6	<0.001
U albumin/creatinine *mg/g*	2210.5 ± 1905.8	1984.4 ± 1744.5	1810.6 ± 1735.4	1290.7 ± 1259.2	<0.001
Serum triglyceride *mg/dL*	191.2 ± 156.2	206.5 ± 158.4	247.0 ± 250.9	235.8 ± 188.8	<0.001
Total cholesterol *mg/dL*	230.3 ± 58.4	230.1 ± 56.3	228.5 ± 56.5	223.6 ± 51.6	0.089
LDL *mg/dL*	146.7 ± 49.0	142.7 ± 45.7	139.4 ± 44.8	139.2 ± 42.9	0.020
Smoking no. (%)	52 (14.1)	66 (18.0)	58 (17.2)	89 (22.8)	0.003
BMI *kg/m²*	29.3 ± 7.3	30.3 ± 6.5	29.3 ± 5.7	30.1 ± 5.6	0.268
Medical history[d]					
Amputation no. (%)	33 (8.9)	34 (9.2)	27 (8.0)	38 (9.7)	0.800
Angina no. (%)	32 (8.6)	33 (9.0)	38 (11.3)	33 (8.4)	0.854
Congestive heart failure no. (%)	22 (5.9)	24 (6.5)	15 (4.5)	17 (4.3)	0.209
Myocardial infarction no. (%)	34 (9.1)	46 (12.5)	52 (15.4)	57 (14.6)	0.014
Neuropathy no. (%)	216 (58.1)	181 (49.2)	161 (47.8)	175 (44.8)	<0.001
Retinopathy no. (%)	286 (76.9)	245 (66.6)	196 (58.2)	210 (53.7)	<0.001

N = Number of participants with measured hemoglobin concentration at baseline.

[a]Plus-minus values are mean ± standard deviation for continuous variables. Number and percent are reported for categorical variables. For each variable, the percentages reflect the total number of patients for whom data were available, which may be less than the total number of patients in the quartile in some cases. Hemoglobin quartiles are based on observed and calculated observations.

[b]19 patients with other race were excluded from race due to small sample size.

[c]Linear regression was performed for continuous variables by median of hemoglobin quartile, and Cochran-Armitage test for trend was performed for each categorical variable by hemoglobin quartile. $P < 0.05$ is significant.

[d]Obtained from patient medical history at baseline.

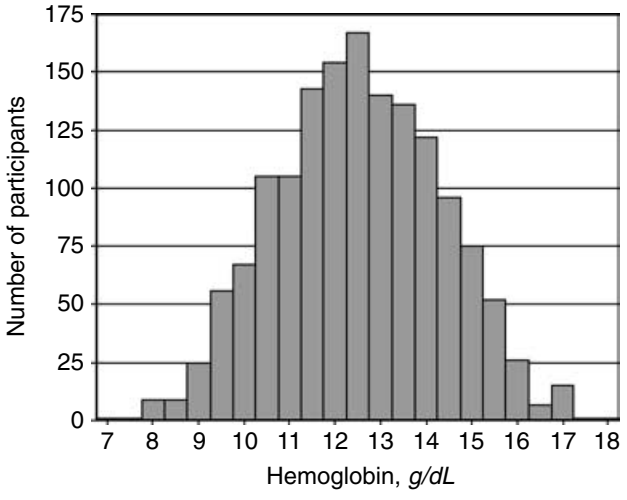


Fig. 1. Hemoglobin distribution at baseline. This histogram illustrates the distribution of hemoglobin at baseline. The y-axis shows the number of participants at each hemoglobin concentration, expressed as frequency. Hemoglobin ranged from 6.8 to 18.0 g/dL, with mean hemoglobin of 12.5 g/dL.

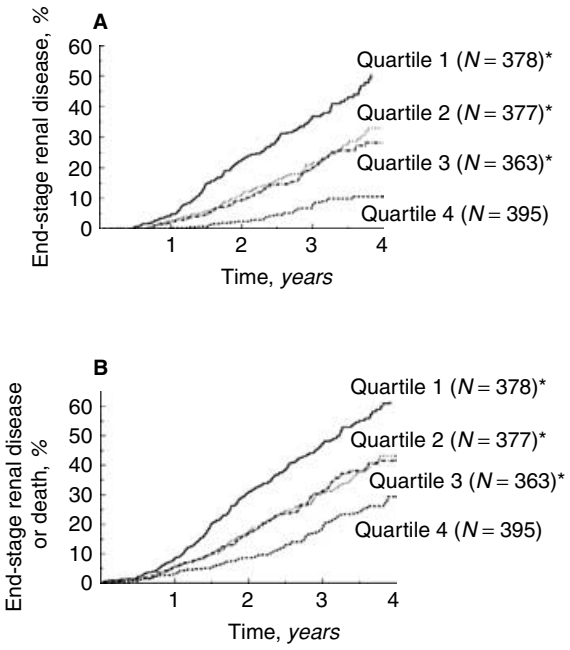
that included estimated glomerular filtration rate did not significantly alter our results, nor did any of the components of baseline medical history.

Because treatment with angiotensin receptor antagonists has been shown to lower hemoglobin in some participants with chronic kidney disease, we conducted an analysis incorporating treatment assignment to losartan into our model in addition to the other prespecified covariates [25, 26]. Although treatment assignment was significant on multivariate analysis, it did not alter our conclusion.

Hemoglobin modeled as a continuous variable was an independent predictor of ESRD with an unadjusted hazard ratio of 0.71 per g/dL of Hb (95% CI 0.67-0.76, $P < 0.001$). The adjusted hazard ratio was 0.90 (95% CI 0.84-0.96, $P = 0.0013$).

End-stage renal disease or death

The proportion of participants reaching the combined end point of ESRD or death over time was significantly



No. at risk					
Quartile 1	378	346	262	125	17
Quartile 2	377	357	311	166	30
Quartile 3	363	343	301	184	22
Quartile 4	395	383	361	247	42
Total	1513	1430	1237	725	115

Fig. 2. Kaplan-Meier curves showing the cumulative incidence of events over the course of the study by quartiles of baseline hemoglobin concentration for ESRD (*A*) and for ESRD or death (*B*). The range of hemoglobin was as follows: 6.8 to 11.3 g/dL (quartile 1), 11.3 to 12.5 g/dL (quartile 2), 12.5 to 13.8 g/dL (quartile 3), and 13.8 to 18.0 g/dL (quartile 4). Cumulative events are shown for each outcome within each hemoglobin quartile. Comparisons are made to quartile 4 (the reference quartile) in each panel. *Indicates $P < 0.05$.

higher for those in the first, second, and third Hb quartiles compared to those in the fourth quartile (Fig. 2). Participants with lower Hb had higher event rates for ESRD or death (Table 2). The event rates for ESRD or death per 1000 patient-years of follow-up were 204.8 for the lowest quartile, 124.9 for the second quartile, 126.4 for the third quartile, and 76.2 for the fourth quartile. The unadjusted hazard ratios for the first, second, and third quartiles were 2.96 (95% CI, 2.32-3.79, $P < 0.001$), 1.72 (95% CI 1.32-2.24, $P < 0.001$) and 1.72 (95% CI 1.32-2.24, $P < 0.001$), compared to the fourth quartile. In the unadjusted analysis, the hazard ratios for participants in the first, second, and third Hb quartiles were significantly greater than the hazard ratio for the fourth quartile. After adjusting for covariates, the hazard ratios for participants in the first, second, and third Hb quartiles were lower in magnitude, but still greater compared to the fourth Hb quartile. The hazard ratios for participants in the first Hb quartile remained significantly different compared to the fourth quartile, while hazard ratios for the second and third quartiles were no longer significantly different from the fourth quartile (Table 2). Adding estimated glomerular filtration rate or baseline medical history or losartan use to the prespecified covariates in the multivariate analysis did not change our conclusion.

Hemoglobin modeled as a continuous variable was an independent predictor of ESRD or death with an unadjusted hazard ratio of 0.80 (95% CI 0.76-0.84, $P < 0.001$). The adjusted hazard ratio was 0.95 (95% CI 0.90-0.99, $P = 0.035$).

DISCUSSION

The principal new finding in this study is that anemia is an independent predictor for progression to ESRD in type 2 diabetics with nephropathy. The significant association between baseline hemoglobin concentration and progression to ESRD was present both when hemoglobin was analyzed by quartiles and as a continuous variable. After adjustment for risk factors for progression of nephropathy, the risk for ESRD was increased even at modest reduction in Hb, that is, below 13.8 g/dL. Moreover, the risk for ESRD nearly doubled (HR 1.99) for participants in the lowest Hb quartile (<11.8 g/dL) compared to those in the highest Hb quartile (≥13.8 g/dL). The adjusted relative risk for ESRD increased by 11% for each 1 g/dL decline in Hb. We also found that the risk for the combined end point of ESRD or death was significantly greater for participants in the lowest Hb quartile, compared to those in the highest Hb quartile. The adjusted relative risk for ESRD or death increased by 5.6% for each 1 g/dL decline in Hb.

A key question is whether the association of low hemoglobin concentration at baseline and subsequent development of ESRD or death is caused by low hemoglobin or low glomerular filtration rate. Our analysis cannot establish causality. However, we controlled for level of renal function in two ways. First, we conducted a multivariate analysis using serum creatinine as a marker of renal function. In this analysis, Hb was an independent risk factor for ESRD and the combined end point of ESRD or death. In a separate model in which we used estimated glomerular filtration rate as a measure of renal function at baseline, we found the same result. Hemoglobin concentration at baseline was an independent predictor of ESRD and the risk for ESRD was increased even at a modest reduction in hemoglobin concentration.

We also conducted a separate analysis that included components of medical history at baseline as covariates. Medical history at baseline was collected based on patient recall and physician input, and also at times, chart review. Given the variability of the sources of information, it is likely that these histories were not collected as carefully

Table 2. Event rates and hazard ratios for ESRD and ESRD or death by hemoglobin quartiles

		ESRD						ESRD or Death					
		Event		Unadjusted		Adjusted[a]		Event		Unadjusted		Adjusted[a]	
Hb Quartile Hb (g/dL)	*N*	No.	Rate[b]	HR (95% CI)	*P* value	HR (95% CI)	*P* value	No.	Rate[b]	HR (95% CI)	*P* value	HR (95% CI)	*P* value
1 <11.3	378	138	144.2	5.87 (4.03-8.55)	<0.001	1.99 (1.34-2.95)	0.001	196	204.8	2.96 (2.32-3.79)	<0.001	1.36 (1.05-1.78)	0.021
2 ≥11.3 –12.5	377	90	84.5	3.25 (2.19-4.82)	<0.001	1.61 (1.08-2.41)	0.020	133	124.9	1.72 (1.32-2.24)	<0.001	1.05 (0.80-1.38)	0.717
3 ≥12.5 – 13.8	363	79	75.6	2.86 (1.91-4.28)	<0.001	1.87 (1.25-2.80)	0.002	132	126.4	1.72 (1.32-2.24)	<0.001	1.29 (0.99-1.69)	0.058
4 ≥13.8	395	34	27.6	1.00	–	1.00	–	94	76.2	1.00	–	1.00	–

HR, hazard ratio.
[a] Adjusted analysis included covariates that were selected from the following variables: age, sex, race, BMI, smoking, systolic and diastolic BP, SCr, albumin, calcium, phosphorus, total cholesterol, LDL, log(triglycerides), and log(urine albumin/creatinine ratio).
[b] Event rate: Event Rate expressed in terms of 1000 patient-years at risk.

as outcomes data during the RENAAL study (i.e., adjudicated by an outcomes committee). Therefore, components of medical history at baseline were not considered in the primary analysis along with baseline characteristics.

We constructed a multivariate model that included treatment assignment to losartan as well. This additional model was constructed to evaluate the possibility that losartan assignment would influence the results of our analysis. By definition, baseline data were collected prior to randomization to drug or placebo. In the additional multivariate model that included treatment assignment to losartan, our conclusion regarding hemoglobin concentration was the same. Therefore, treatment with losartan does not appear to be the explanation for the relationship between baseline Hb and ESRD, or the combined end point of ESRD or death in our analysis. As noted above, adjustment for treatment assignment to losartan did not alter the relationship between Hb and outcomes in our study. Losartan use was balanced in each quartile. Other investigators have shown that angiotensin receptor blockers can cause anemia [26]. Both losartan and placebo groups had a mean hemoglobin concentration of 12.5 g/dL at baseline, and lower mean hemoglobin concentration at 36 months: 11.9 g/dL in the losartan group and 12.3 g/dL in the placebo group.

Gender is significantly associated with each end point in univariate analysis. Lower hemoglobin in adult women has been well described, and in RENAAL, women had greater proteinuria than men. Therefore, in the multivariate analysis, when adjusting for both proteinuria and hemoglobin, the significance of gender becomes much weaker. After adjusting for significant risk factors, the risk across Hb quartiles is similar for men and for women.

Previous investigations examining the potential impact of anemia on progression of renal disease were limited by heterogeneous causes of kidney disease, small numbers of participants, short duration of follow-up, and lack of well-defined end points [8–17]. Moreover, none of these studies examined the impact of anemia on ESRD; rather, these studies utilized outcomes such as serum creatinine, the inverse of serum creatinine, and clearance of iothalamate, inulin, or para-amino hippuric acid. Our study controlled for proteinuria and other factors associated with progression of nephropathy. Furthermore, we evaluated the association between anemia and ESRD in a well-defined population of 1513 participants with type 2 diabetes and nephropathy followed for an average for 3.4 years, the longest renal end point trial to date. Our patient population of patients with type 2 diabetes and nephropathy reflects a far more homogeneous disease process than in prior studies. Our findings demonstrate that higher baseline hemoglobin was independently associated with longer time to ESRD in participants with type 2 diabetes and nephropathy.

Anemia may begin in early stages of CKD [27, 28]. Furthermore, the number of individuals with CKD in the United States is currently estimated at 19.2 million [27]. Moderate anemia (hematocrit <30) at incident dialysis is associated with higher hospitalization rates, cost of care [29, 30], and increased mortality [31]. Anemia is also a major risk factor for cardiac morbidity and mortality for participants with CKD [2, 3, 4, 6, 22, 31, 32–35]. In fact, congestive heart failure (CHF) is the leading cause of cardiac morbidity and mortality in diabetics, and anemia and CHF coexist in the CKD population [1, 36, 37].

Treatment of anemia with erythropoietin improves quality of life, reduces risk of progression to left ventricular hypertrophy in predialysis CKD patients, and decreases hospitalization in ESRD [6, 7, 38]. However, less than 30% of anemic pre-ESRD patients are treated for anemia [39–41]. Because only 18 of 1513 participants in our study were receiving erythropoietin, we were unable to ascertain the impact of correction of anemia with erythropoietin on ESRD and death outcomes. This low rate of anemia treatment reflects uncommon use of this drug during the time of the study (before 2001).

Current National Kidney Foundation guidelines for pre-ESRD participants recommend treatment to a goal Hb of 11 to 12 g/dL [42]. Our data suggest that even mild anemia, Hb <13.8 g/dL, increases risk for progression to ESRD. Whether treatment or prevention of anemia may improve outcomes among those with type 2 diabetes and nephropathy remains to be determined. Current trials of anemia treatment in CKD are following patients for cardiovascular and renal outcomes (Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin beta, Correction of Hemoglobin and Outcomes In Renal Insufficiency, Trial to Reduce Cardiovascular Events with Aranesp) [43–45].

The mechanism by which anemia might increase risk for ESRD in diabetic nephropathy is unknown. Anemia could aggravate fibrosis in the failing kidney by causing renal tissue hypoxia. Renal hypoxia is known to stimulate cytokines such as hypoxia inducing factor-1, which contribute to renal scarring [46]. In addition, hypoxia stimulates renal sympathetic activity, resulting in reduction in renal blood flow and glomerular filtration rate over time [47]. Finally, anemia may induce or exacerbate underlying cardiac disease, which may in turn lead to worsening renal function and hasten ESRD. Additional studies to evaluate these or other mechanisms in participants with nephropathy and diabetes are needed to determine how anemia contributes to progression of kidney disease to end-stage.

Limitations to our study include the fact that the RENAAL study was not specifically designed to evaluate the impact of anemia on progression of nephropathy. The clear and consistent relationship between hemoglobin and renal end points in the present study cannot prove cause and effect. However, our findings add to those of others who suggest that anemia may contribute to progression of CKD [8], and that decreasing kidney function is associated with a higher prevalence of anemia [48]. In addition, our analysis could not evaluate the mechanism of anemia in participants with progression in diabetic nephropathy. Laboratory data regarding other factors related to anemia, such as nutritional deficiencies of iron, B12, or folate, iron saturation, red blood cell survival, hemoglobin electrophoresis, expression of hypoxia-inducible factor, and toxic metabolites of uremia were not obtained. Furthermore, specific anemia-related history, such as hemolysis, menorrhagia, or gastrointestinal hemorrhage was not collected. We cannot exclude the possibility that an unmeasured additional confounding variable may explain our observation. Still, we adjusted for risk factors for progression of diabetic nephropathy, as well as preexisting cardiovascular disease and treatment assignment to losartan.

CONCLUSION

Our data identify mild anemia (Hb <13.8 g/dL) as an independent predictor of ESRD in type 2 diabetics with nephropathy, and more severe anemia (Hb <11.3 g/dL) as a predictor of ESRD or death. Because anemia in patients with diabetic nephropathy is a modifiable risk factor and can be treated and theoretically prevented by administration of erythropoietin, further studies on the relationship between anemia and progression of kidney disease in patients with type 2 diabetes are warranted.

ACKNOWLEDGMENTS

We are indebted to Denise R. Ramjit and Roger L. Simpson for their substantial contribution. This study was supported by the United States National Institutes of Health grants numbers T32 DK 07257–20, 1 F32-DK62687-01, and 5-K-24 02818–02.

Reprint requests to Robert Toto, M.D., Division of Nephrology, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390–8856.
E-mail: Robert.Toto@UTSouthwestern.edu

REFERENCES

1. U.S. Renal Data System, USRDS 2002 Annual Data Report: *Atlas of End-Stage Renal Disease in the United States*, Bethesda, MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2002
2. Ma JZ, Ebben J, Xia H, Collins AJ: Hematocrit level and associated mortality in hemodialysis patients. *J Am Soc Nephrol* 10(3):610–619, 1999
3. Foley RN, Parfrey PS, Sarnak MJ: Epidemiology of cardiovascular disease in chronic renal disease. *J Am Soc Nephrol* 9(12 Suppl):S16–23, 1998
4. Foley RN, Parfrey PS, Kent GM, *et al*: Serial change in echocardiographic parameters and cardiac failure in end-stage renal disease. *J Am Soc Nephrol* 11(5):912–916, 2000
5. Harnett JD, Foley RN, Kent GM, *et al*: Congestive heart failure in dialysis patients: Prevalence, incidence, prognosis and risk factors. *Kidney Int* 47(3):884–890, 1995

6. Levin A, Thompson CR, Ethier J, *et al*: Left ventricular mass index increase in early renal disease: Impact of decline in hemoglobin. *Am J Kidney Dis* 34(1):125–134, 1999
7. Silberberg J, Racine N, Barre P, Sniderman AD: Regression of left ventricular hypertrophy in dialysis patients following correction of anemia with recombinant human erythropoietin. *Can J Cardiol* 6(1):1–4, 1990
8. Kuriyama S, Tomonari H, Yoshida H, *et al*: Reversal of anemia by erythropoietin therapy retards the progression of chronic renal failure, especially in nondiabetic patients. *Nephron* 77(2):176–185, 1997
9. The U.S. Recombinant Human Erythropoietin Predialysis Study Group: Double-blind, placebo-controlled study of the therapeutic use of recombinant human erythropoietin for anemia associated with chronic renal failure in predialysis patients. *Am J Kidney Dis* 18(1):50–59, 1991
10. Austrian Multicenter Study Group of r-HuEPO in Predialysis Patients: Effectiveness and safety of recombinant human erythropoietin in predialysis patients. *Nephron* 61(4):399–403, 1992
11. Eschbach JW, Kelly MR, Haley NR, *et al*: Treatment of the anemia of progressive renal failure with recombinant human erythropoietin. *N Engl J Med* 321(3):158–163, 1989
12. Abraham PA, Opsahl JA, Rachael KM, *et al*: Renal function during erythropoietin therapy for anemia in predialysis chronic renal failure patients. *Am J Nephrol* 10(2):128–136, 1990
13. Stone WJ, Graber SE, Krantz SB, *et al*: Treatment of the anemia of predialysis patients with recombinant human erythropoietin: A randomized, placebo-controlled trial. *Am J Med Sci* 296(3):171–179, 1988
14. Watson AJ, Gimenez LF, Cotton S, *et al*: Treatment of the anemia of chronic renal failure with subcutaneous recombinant human erythropoietin. *Am J Med* 89(4):432–435, 1990
15. Roth D, Smith RD, Schulman G, *et al*: Effects of recombinant human erythropoietin on renal function in chronic renal failure predialysis patients. *Am J Kidney Dis* 24(5):777–784, 1994
16. Kleinman KS, Schweitzer SU, Perdue ST, *et al*: The use of recombinant human erythropoietin in the correction of anemia in predialysis patients and its effect on renal function: A double-blind, placebo-controlled trial. *Am J Kidney Dis* 14(6):486–495, 1989
17. Jungers PY, Choukroun G, Oualim Z, *et al*: Beneficial influence of recombinant human erythropoietin therapy on the rate of progression of chronic renal failure in predialysis patients. *Nephrol Dial Transplant* 16(2):307–312, 2001
18. Keane WF, Brenner BM, De Zeeuw D, *et al*: The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: The RENAAL study. *Kidney Int* 63(4):1499–1507, 2003
19. Brenner BM, Cooper ME, de Zeeuw D, *et al*: The losartan renal protection study–rationale, study design and baseline characteristics of RENAAL (reduction of endpoints in NIDDM with the angiotensin II antagonist losartan). *Journal of the Renin-Angiotensin-Aldosterone System* 1(4):328–335, 2000
20. Brenner BM, Cooper ME, de Zeeuw D, *et al*: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345(12):861–869, 2001
21. Eknoyan G, Levin NW: K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification—foreword. *Am J Kidney Dis* 39(2):S14–S266, 2002
22. Gilbertson DT, Li SL, Murray A, *et al*: The competing risks of death vs. ESRD in Medicare beneficiaries 65+ with chronic kidney disease, CHF, and anemia. *J Am Soc Nephrol* 13:439A–439A, 2002
23. Adler AI, Stevens RJ, Manley SE, *et al*: Development and progression of nephropathy in type 2 diabetes: The United Kingdom prospective diabetes study (UKPDS 64). *Kidney Int* 63(1):225–232, 2003
24. Armitage P: Tests for linear trends in proportions and frequencies. *Biometrics*11:375–386, 1955
25. Schwarzbeck A, Wittenmeier KW, Hallfritzsch U: Anaemia in dialysis patients as a side-effect of sartanes. *Lancet* 352(9124):286, 1998
26. Stigant CE, Cohen J, Vivera M, Zaltzman JS: ACE inhibitors and angiotensin II antagonists in renal transplantation: An analysis of safety and efficacy. *Am J Kidney Dis* 35(1):58–63, 2000
27. Coresh J, Astor BC, Greene T, *et al*: Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third national health and nutrition examination survey. *Am J Kidney Dis* 41(1):1–12, 2003
28. Obrador GT, Ruthazer R, Arora P, *et al*: Prevalence of and factors associated with suboptimal care before initiation of dialysis in the United States. *J Am Soc Nephrol* 10(8):1793–1800, 1999
29. St Peter WL, Xue J, Ebben J, *et al*: Pre-ESRD erythropoietin use predicts Medicare expenditures in the periods before & after ESRD diagnosis. *J Am Soc Nephrol* 12:247A–247A, 2001
30. Li SY, Collins AJ: Higher hematocrit values are consistently associated with lower morbidity under NKF DOQI guidelines in incident hemodialysis patients. *J Am Soc Nephrol* 13:221A–221A, 2002
31. Xue JL, St Peter WL, Ebben JP, *et al*: Anemia treatment in the pre-ESRD period and associated mortality in elderly patients. *Am J Kidney Dis* 40(6):1153–1161, 2002
32. Xue JL, Ebben J, St Peter WL, Collins AJ: An association of pre-end-stage renal disease epoetin treatment with post-ESRD mortality. *Am J Kidney Dis* 37(4):98, 2001
33. Herzog CA, Ma JZ, Collins AJ: Poor long-term survival after acute myocardial infarction among patients on long-term dialysis. *N Engl J Med* 339(12):799–805, 1998
34. Levin A, Singer J, Thompson CR, *et al*: Prevalent left ventricular hypertrophy in the predialysis population: Identifying opportunities for intervention. *Am J Kidney Dis* 27(3):347–354, 1996
35. Middleton RJ, Parfrey PS, Foley RN: Left ventricular hypertrophy in the renal patient. *J Am Soc Nephrol* 12(5):1079–1084, 2001
36. Kannel WB, McGee DL: Diabetes and cardiovascular disease. The Framingham study. *JAMA* 241(19):2035–2038, 1979
37. Xue JL, Frazier ET, Everson SE, *et al*: Diabetes, hypertension and heart disease in patients starting renal replacement therapy. *J Am Soc Nephrol* 13:443A–444A, 2002
38. Mix C, St Peter WL, Ebben J, *et al*: Hospitalization in chronic kidney disease (CKD). *J Am Soc Nephrol* 13:617A–618A, 2002
39. Kazmi WH, Kausz AT, Khan S, *et al*: Anemia: An early complication of chronic renal insufficiency. *Am J Kidney Dis* 38(4):803–812, 2001
40. Fink J, Blahut S, Reddy M, Light P: Use of erythropoietin before the initiation of dialysis and its impact on mortality. *Am J Kidney Dis* 37(2):348–355, 2001
41. Obrador GT, Roberts T, St Peter WL, *et al*: Trends in anemia at initiation of dialysis in the United States. *Kidney Int* 60(5):1875–1884, 2001
42. National Kidney Foundation: K/DOQI clinical practice guidelines for anemia of chronic kidney disease: Update 2000. *Am J Kidney Dis* 37(Suppl 1):S182–238, 2001
43. Eckardt KU: Cardiovascular risk Reduction by Early Anemia Treatment with Epoetin Beta (CREATE) Trial: The CREATE trial—building the evidence. *Nephrol Dial Transplant* 16(Suppl 2):16–18, 2001
44. Reddan D, Tran LL, Jollis J, Singh A: Anemia correction and left ventricular hypertrophy: An echocardiographic substudy of the correction of hemoglobin and outcomes in renal insufficiency (CHOIR) study. *J Am Soc Nephrol* 13:676A–676A, 2002
45. Rao M, Pereira BJG: Prospective trials on anemia of chronic disease: The Trial to Reduce Cardiovascular Events with Aranesp® Therapy (TREAT). *Kidney Int* 64(Suppl 87):12–19, 2003
46. Rosenberger C, Mandriota S, Jurgensen JS, *et al*: Expression of hypoxia-inducible factor-1alpha and -2alpha in hypoxic and ischemic rat kidneys. *J Am Soc Nephrol* 13(7):1721–1732, 2002
47. Denton KM, Shweta A, Anderson WP: Preglomerular and postglomerular resistance responses to different levels of sympathetic activation by hypoxia. *J Am Soc Nephrol* 13(1):27–34, 2002
48. Hsu CY, McCulloch CE, Curhan GC: Epidemiology of anemia associated with chronic renal insufficiency among adults in the United States: Results from the third national health and nutrition examination survey. *J Am Soc Nephrol* 13(2):504–510, 2002