

## Hemoglobin Variability and Mortality in ESRD

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### ABSTRACT

Hemoglobin levels vary substantially over time in hemodialysis patients, and this variability may portend poor outcomes. For a given patient, hemoglobin concentration over time can be described by absolute levels, rate of change, or by the difference between observed level and expected level based on the preceding trend (*i.e.*, seemingly random variability). We investigated the independent associations of these different methods of describing hemoglobin over time with mortality in a retrospective cohort of 34,963 hemodialysis patients. Hemoglobin concentration over time was modeled with linear regression for each subject, and the model was then used to define the subject's absolute level of hemoglobin (intercept), temporal trend in hemoglobin (slope), and hemoglobin variability (residual standard deviation). Survival analyses indicated that each 1g/dl increase in the residual standard deviation was associated with a 33% increase in rate of death, even after adjusting for multiple covariates. Patient characteristics accounted for very little of the variation in our hemoglobin variability metric ( $R^2 = 0.019$ ). We conclude that greater hemoglobin variability is independently associated with higher mortality.

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Widespread use of erythropoiesis-stimulating agents and intravenous iron has revolutionized anemia management in ESRD. Over the past 15 yr, average hemoglobin levels have increased from <10 g/dl to >12 g/dl among chronic dialysis patients.<sup>1</sup> However, these higher levels of hemoglobin have not necessarily translated into improved patient outcomes.

Multiple studies suggest an association between higher hemoglobin level and improved quality of life, physical function, and exercise capacity,<sup>2–5</sup> but the association with survival is less clear. Whereas observational studies have generally shown increased survival with higher hemoglobin levels,<sup>6–9</sup> randomized trials have not shown such benefits.<sup>10–12</sup> Moreover, some randomized trials have suggested an increase in cardiovascular events associated with higher hemoglobin levels among hemodialysis<sup>5,10</sup> and chronic kidney disease patients.<sup>11,12</sup> The evidence from randomized trials is especially significant in light of a recent report showing that for-profit dialysis chains administer higher epoetin doses even when hemoglobin is within

the 11 to 12 g/dl range, and have a higher proportion of patients with hemoglobin >12 g/dl.<sup>13,14</sup>

Preliminary evidence suggests the effects of hemoglobin on clinical outcomes may not be captured by the absolute level of hemoglobin alone. Measures of their change over time have also been associated with adverse patient outcomes.<sup>15,16</sup> Such changes may take the form of temporal trends or random variability about this trend (henceforth referred to as hemoglobin variability or Hb-Var). Prior studies examining the association between hemoglobin levels and out-

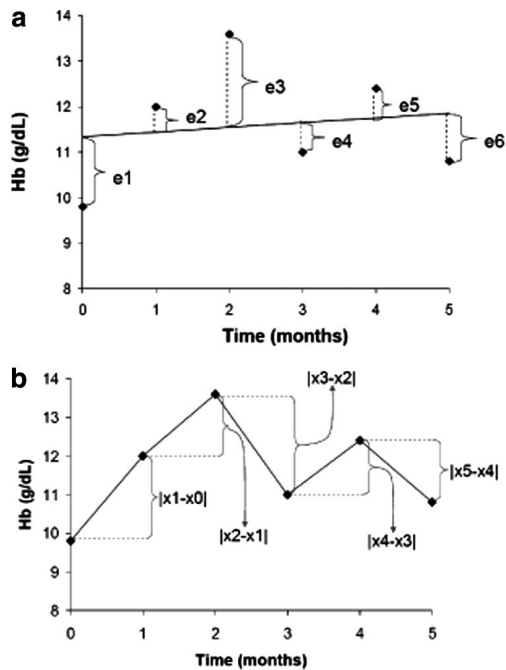
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**Figure 1.** (a) Definition of regression-based hemoglobin parameters. For each subject, observed hemoglobin values were fit to a linear regression model  $Hb(i) = a + b \times \text{month}(i) + \epsilon(i)$ ,  $i = 0, 1, 2, \dots, 5$ : Absolute hemoglobin level was defined as the intercept, temporal trend as the slope and Hb-Var as the residual SD (roughly, the square root of the average squared vertical distance between observed hemoglobin levels and the regression line [e0 through e5]) parameter estimates from these models. (b) Average absolute hemoglobin change. This alternative metric of Hb-Var was defined as the average of the vertical distance between successive points (e.g.  $x_1 - x_0$ ,  $x_2 - x_1$ ,  $x_3 - x_2$ ,  $x_4 - x_3$ ,  $x_5 - x_4$ ).

comes have not fully distinguished among absolute hemoglobin level, temporal trend, and Hb-Var. This may have contributed to inconsistent findings across studies, and made it difficult to determine the independent relationship between each of these aspects of hemoglobin management and outcome.<sup>16–20</sup>

To better elucidate the independent association between Hb-Var and outcome, we have developed a new, regression-based metric of Hb-Var that simultaneously describes and distinguishes among variability, absolute level of, and temporal trend in longitudinal hemoglobin levels (Figure 1a). Using a historical national cohort of prevalent hemodialysis patients, we compare this regression-based measure of Hb-Var to an alternative formulation (the average absolute change; Figure 1b),<sup>21</sup> explore clinical predictors of high levels of Hb-Var, and characterize the association between Hb-Var and all-cause mortality.

## RESULTS

### Subject Eligibility and Characteristics

The Fresenius Medical Care (FMC) database included records on 34,963 hemodialysis patients: 30,693 had at least two hemo-

**Table 1.** Baseline demographic characteristics, laboratory and treatment-related measures in the cohort

Age, yr (mean $\pm$ SD)	60.1 $\pm$ 15.1
Duration of ESRD, yr (mean $\pm$ SD)	3.6 $\pm$ 3.6
Gender	
male, %	50.6
female, %	49.4
Race, %	
white	48.9
black	44.7
Native American	0.7
Asian/Pacific Islander	1.6
other	4.2
Diabetes mellitus, %	
adult onset	40.5
juvenile onset	4.5
none	55
Laboratory measures at 3 mo (mean $\pm$ SD)	
hemoglobin, g/dl	10.2 $\pm$ 1.2
urea reduction ratio, %	67.3 $\pm$ 7.0
Kt/V	1.4 $\pm$ 0.2
albumin, g/dl	3.9 $\pm$ 0.4
bicarbonate, mEq/L	20.6 $\pm$ 3.0
aspartate aminotransferase, IU/L	19 $\pm$ 15.5
calcium, mg/dl	9.0 $\pm$ 0.8
phosphate, mg/dl	6.0 $\pm$ 1.7
intact PTH, pg/ml	315 $\pm$ 433
ferritin, ng/ml	352 $\pm$ 402
transferrin saturation, %	26.7 $\pm$ 11.6
iron level, $\mu$ g/dl	57.2 $\pm$ 24.3
Treatment measures (mean $\pm$ SD)	
intravenous iron dose summed over 3 mo, mg	944 $\pm$ 546
erythropoietin dose average over 3 mo, U	50,875 $\pm$ 32,238

*N* = 34,963 patients.

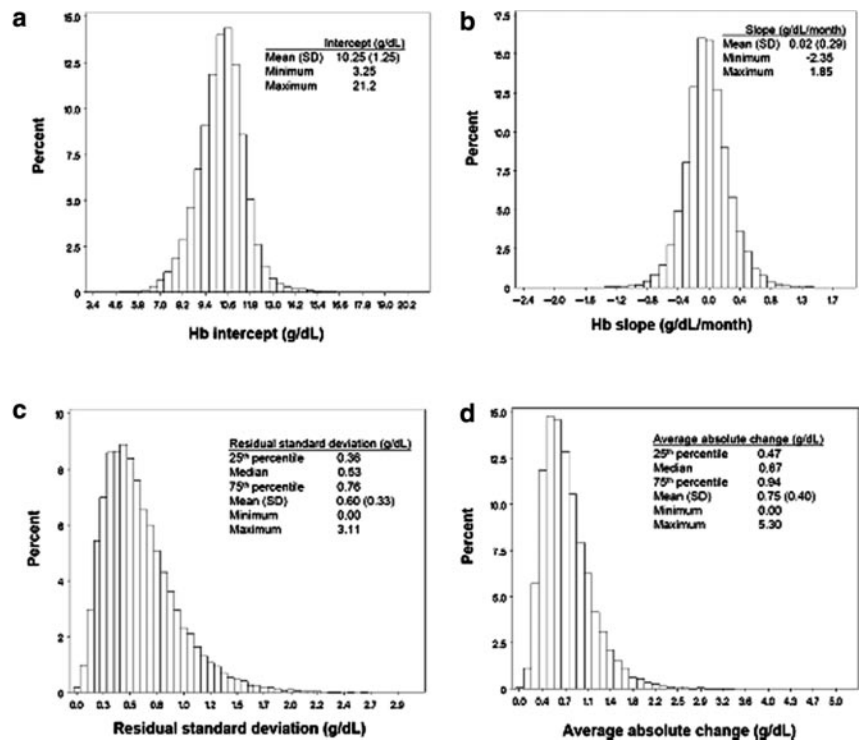
globin measurements during the exposure window needed to measure the average absolute change in hemoglobin, and 30,603 had at least three hemoglobin measurements needed to define residual SD. The primary survival analysis considered the 19,150 subjects who remained enrolled throughout the hemoglobin exposure window and had no missing hemoglobin or covariates values over that period; 8797, 5246, and 2523 subjects qualified for sensitivity analyses that further restricted consideration to subjects with minimum hemoglobin  $\geq 9.5$  g/dl,  $\geq 10.0$  g/dl, and  $\geq 10.5$  g/dl, respectively.

A description of the cohort can be found in Table 1. Among the 34,963 patients eligible for the study, 26.2% died, 28.5% transferred care or were lost to follow-up, and 45.3% were being followed at end of study.

Absolute hemoglobin level (intercept) and temporal trend in hemoglobin (slope) were normally distributed across the cohort (Figure 2a and b). Hemoglobin variability defined as Res-SD and as average absolute change demonstrated a right skewed distribution (Figure 2c, and d).

### Relationship between Metrics of Hemoglobin Variability and Other Hemoglobin Parameters

In subjects for whom both Hb-Var measures could be defined,



**Figure 2.** Distribution of the absolute level of hemoglobin (intercept, a), temporal hemoglobin trend (slope, b), and hemoglobin variability (residual SD, c) as measured via our regression-based approach. The distribution of hemoglobin variability as defined by the average absolute change is displayed (d).

the average absolute change and residual SD were highly correlated ( $r = 0.91$ ). There was little correlation between intercept and Hb-Var, as defined as either Res-SD ( $r = -0.10$ ) or average absolute change ( $r = -0.08$ ). These near-zero values indicate that neither metric of Hb-Var was explained well by absolute hemoglobin level.

Both measures of Hb-Var demonstrated low degrees of correlation with temporal hemoglobin trend ( $r = 0.01$  and  $r = 0.03$ , respectively). However, scatter plots of slope *versus* average absolute change, but not residual SD, demonstrated a conical shape (Figure 3), indicating dependence of average absolute change on temporal hemoglobin trend (*i.e.*, the values of average absolute change could not be small when temporal hemoglobin change was large). Therefore, we chose Res-SD as our primary metric of Hb-Var in subsequent analyses.

The distribution of Res-SD was similar across strata of patients defined by hemoglobin trend: Mean Res-SD was 0.64 g/dl, 0.55 g/dl, and 0.64 g/dl for subjects with slope  $< -1$  g/dl per 6 mo,  $> -1$  and  $< 1$  g/dl per 6 mo,  $> 1$  g/dl per 6 mo (corresponding to what we hypothesized was a meaningful clinical temporal change).

### Prediction of Residual SD of Hemoglobin

A linear regression model was used to identify potential predictors of increased Hb-Var. Although the variables of age, gender, duration of ESRD, average hemoglobin level, serum albumin, aspartate aminotransferase, calcium, intact parathyroid hormone level, iron level, and erythropoietin dose reached conventional levels of statistical significance (possibly because of a large sample size), the overall  $R^2$  for this model was

0.019, demonstrating that these factors, collectively, accounted for very little of observed variation in Hb-Var among subjects.

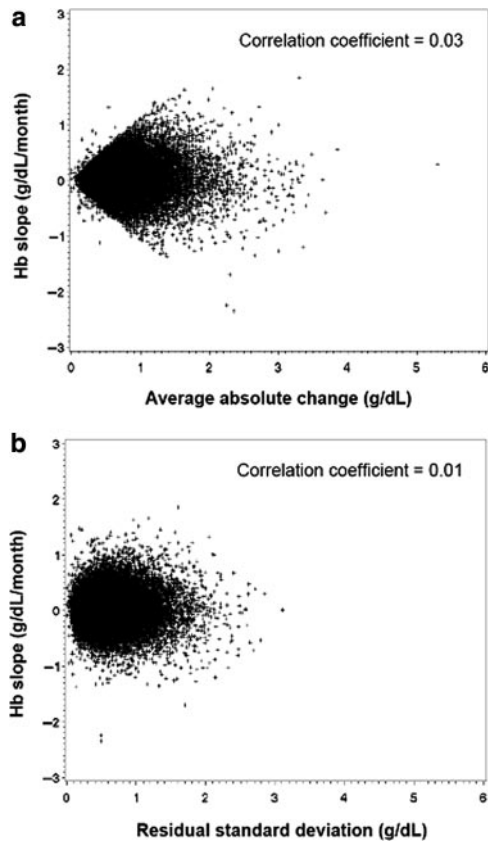
### Survival Analysis

Hb-Var was associated with decreased survival after adjusting for known confounders in adjusted proportional hazards analysis. For each 1-g/dl increase in hemoglobin Res-SD, there was a 33% increase in the rate of death (Table 2). As expected, a higher absolute level of hemoglobin and a positive value for temporal trend in hemoglobin were also associated with longer survival. There were no significant interactions between Hb-Var and absolute level of hemoglobin, or between Hb-Var and temporal trend in hemoglobin (data not shown).

### Sensitivity Analyses

Three subgroups of patients with increasingly restrictive minimum hemoglobin values during the 6-mo hemoglobin exposure windows were examined. For each subgroup, the association between Hb-Var and survival was of the same magnitude and in the same direction as the overall population except for the highest minimum hemoglobin group, for which the confidence intervals were wide (Table 3) because of the small sample size.

Finally, while beyond the scope of this article, we have also assessed the independent association between greater Hb-Var and increased all-cause mortality by fitting history-adjusted marginal structural models as yet another method to control for time-dependent confounding.<sup>22–24</sup> This analysis yielded nearly identical magnitudes of association between hemoglobin variability and mortality (data not shown).



**Figure 3.** Scatter plots of slope versus average absolute change (a) and slope versus residual SD (b). The conical shape of slope versus average absolute change demonstrates the interdependence of this metric of hemoglobin variability on temporal hemoglobin trend, which is largely abrogated for residual SD.

**Table 2.** Association between hemoglobin exposure parameters and mortality\*

Exposure	HR (95% CI)
Absolute level of hemoglobin (per g/dl)	0.81 (0.77 to 0.84)
Temporal trend in hemoglobin (per g/dl per mo)	0.51 (0.44 to 0.59)
Hemoglobin variability	
per 0.50 g/dl	1.15 (1.10 to 1.20)
per 0.75 g/dl	1.24 (1.16 to 1.32)
per 1.00 g/dl	1.33 (1.22 to 1.45)
per 1.50 g/dl	1.53 (1.35 to 1.75)

\*Adjusted for variables in Table 1 and for one another. Survival analysis ( $n = 19,150$ ); deaths = 4536.

## DISCUSSION

Variability of hemoglobin over time both complicates management of anemia in ESRD, and is potentially associated with increased mortality among dialysis-treated patients. Reported measures of hemoglobin variability have not characterized variability distinct from the temporal trend in hemoglobin.<sup>15,17–20</sup> We established that the hemoglobin variability, as measured by the residual SD of hemoglobin, was independent

of both the absolute level and trend over time in hemoglobin concentration, and was superior in this regard to average absolute change. Although we were unable to identify patient demographic, laboratory, or treatment-related characteristics that explained much of the observed variability in Hb-Var, we demonstrated a strong and independent association between high levels of Hb-Var and greater mortality.

Survival analysis demonstrated a 33% increase in the adjusted rate of death for each 1-g/dl increase in Hb-Var. Greater Hb-Var was strongly associated with diminished survival, even after multivariable adjustment for absolute level and temporal trend in hemoglobin concentration among ESRD patients receiving chronic hemodialysis. This finding was also consistent within all but one subgroup with restricted minimum levels of hemoglobin, in which the opportunity for residual confounding was less plausible.

The consistency of results across various modeling strategies demonstrating greater hemoglobin variability associated with increased mortality supports the hypothesis that Hb-Var represents an important physiologic stress. Other than in chronic diseases such as ESRD, hemoglobin levels are typically maintained within a narrow range, ensuring consistent tissue oxygen delivery. Repeated episodes of relative ischemia and tissue hypoxia in ESRD may result in organ dysfunction or injury. The myocardium may be particularly vulnerable to hemoglobin variability, as it compensates for periods of reduced oxygen delivery with increased output and myocardial cell growth.<sup>25</sup> The results may be repeated activation and resetting of cardiac growth signals, and the development of pathologic changes such as left ventricular dilation or hypertrophy.<sup>26,27</sup> The autonomic nervous system may also be vulnerable to hemoglobin variability, as autonomic dysfunction has been observed in other conditions that predispose patients to fluctuating hemoglobin levels, such as sickle cell anemia.<sup>28,29</sup> In this population, autonomic dysfunction has been implicated as a putative risk factor for sudden death.<sup>28</sup> Alterations in cardiac and autonomic nervous system function are particularly relevant in ESRD, as cardiovascular morbidity and mortality are very common in this population.<sup>30</sup>

To our knowledge, this is the first report of an association between hemoglobin variability, independent of hemoglobin level and trend, and mortality. A retrospective cohort study of Medicare patients on chronic hemodialysis attempted to explore the association between hemoglobin variability and outcomes, but failed to adequately distinguish between variability, absolute hemoglobin level, and temporal hemoglobin trend.<sup>15–16,18</sup> In that study, subjects were stratified monthly according to hemoglobin level, and subsequently categorized as having stable hemoglobin (remained in the same stratum over 6 mo), low-amplitude hemoglobin change (fluctuated between adjacent strata regardless of direction), or high-amplitude hemoglobin change (fluctuated among all three strata in any order). Patients with stable hemoglobin in the 11 to 12.5 g/dl stratum had lower rates of hospitalization and mortality than patients with high or low amplitude swings. However, the survival benefit to stable hemoglobin was limited to

**Table 3.** Association between hemoglobin exposure parameters and mortality in subgroups of patients defined by restrictive minimum hemoglobin concentrations\*

	Min Hb $\geq 9.5$ (n = 8,797; deaths = 1749)HR (95% CI)	Min Hb $\geq 10$ (n = 5246; deaths = 990)HR (95% CI)	Min Hb $\geq 10.5$ (n = 2523; deaths = 443)HR (95% CI)
Absolute Hb, g/dl	0.92 (0.83 to 1.01)	0.99 (0.87 to 1.14)	1.17 (0.96 to 1.43)
Trend Hb, g/dl per mo	0.89 (0.64 to 1.23)	1.03 (0.65 to 1.63)	1.07 (0.54 to 2.13)
Hb-Var, g/dl	1.64 (1.33 to 2.02)	1.64 (1.22 to 2.22)	1.02 (0.61 to 1.69)

\*Adjusted for covariates and for one another. Min, minimum.

patients within the 11 to 12.5 g/dl stratum.<sup>15,16</sup> Thus, it was unclear if the association between hemoglobin stability and outcomes was attributable to the absence of hemoglobin variability, the absolute hemoglobin level achieved, absence of temporal hemoglobin trend, or some combination of all three.

Numerous studies have examined the association between absolute hemoglobin level and mortality. We observed a 19% reduction in rate of death for each 1-g/dl increase in the level of hemoglobin. This finding is similar in direction and magnitude to findings from previous observational studies.<sup>7–8,31–33</sup> It should be noted that prior studies have demonstrated that the survival benefit derived from higher hemoglobin levels is confined to subjects with hemoglobin levels  $\leq 12$  g/dl.<sup>5,8,10,31–33</sup> This study did not include a sufficient number of subjects with hemoglobin levels  $>12$  g/dl to permit generalization of its findings to this population. Furthermore, the limited number of subjects in this higher range of hemoglobin prevents us from making inferences regarding the upper limit of hemoglobin treatment targets.

One limitation of this study is the possibility that more comorbid illness led to subsequent higher degrees of Hb-Var, partially explaining the relationship we observed between Hb-Var and mortality. To address this possibility, we sought to conduct analyses among subjects with better health status whose Hb-Var would more likely be the result of treatment protocols rather than variations in health status. To accomplish this, we limited all analyses to subjects with no missing hemoglobin values over the exposure period (hypothesizing that such patients missed fewer outpatient dialysis treatments and were, therefore, healthier). In addition, we performed sensitivity analyses among subgroups of subjects with increasingly restrictive minimum hemoglobin levels (e.g. Hb  $\geq 9.5$  g/dl,  $\geq 10$  g/dl, and  $\geq 10.5$  g/dl), under the assumption that these groups had with a lower prevalence of comorbidities that could function as time-dependent confounders. Two of these three subgroup analyses (Hb  $\geq 9.5$  g/dl and Hb  $\geq 10$  g/dl) yielded results consistent with our primary findings. Analysis of the smallest subgroup ( $\geq 10.5$  g/dl) failed to demonstrate an association between Hb-Var and mortality: It is unclear if this disparity resulted from attenuated statistical power, from residual confounding on the basis of comorbidity in the remaining subgroups, or from a true absence of effect of Hb-Var in this subgroup. Preliminary investigation using formal approaches to control for time-dependent confounding, while beyond the scope of this paper, suggests findings consistent with those presented here.<sup>22</sup> As is the case in all observational research, none of these approaches

completely eliminates the possibility of unmeasured confounding nor assures the causal nature of the observed associations.

Another potential limitation of our study is that we evaluated a cohort assembled in 1996, when clinical guidelines and practice patterns were substantially different than guidelines today. For example, mean hemoglobin, monthly erythropoietin, and ferritin levels were lower than those reported in more recent cohorts.<sup>1,8</sup> Future studies involving cohorts with substantial representation of subjects with hemoglobin levels  $>12$  g/dl will be an important next step in examining the association between Hb-Var and survival.

Although current patterns of Hb-Var may differ from those seen in this historical cohort, evidence suggests that substantial Hb-Var still exists among chronic dialysis patients.<sup>8,15,17–20</sup> At least one study documented that, while average levels of hemoglobin increased between 1995 and 2000, the inpatient SD (a measure of hemoglobin variability) remained constant.<sup>20</sup> Furthermore, while changes in practice patterns may affect the degree of Hb-Var seen in dialysis populations, there is no reason to suspect that they would fundamentally alter the relationship between Hb-Var and mortality.

Our study was also limited by the data available to us. In particular, we did not have access to detailed clinical records and were, therefore, only able to include the covariates described in our methods to adjust for confounding. In addition, we had no access to hospitalization data, which prevented us from using such information either as an indication of comorbidity or as a study outcome, *per se*.

Finally, we studied a large cohort of hemodialysis patients who were all treated within one large US dialysis chain, potentially limiting the generalizability to all chronic hemodialysis patients. Nonetheless, this study of  $>30,000$  hemodialysis patients, who represented 20% of US hemodialysis patients in 1996, included a diverse study group that was likely to be largely reflective of the nation's dialysis population.

## Conclusion

We have described a new method of measuring hemoglobin variability that measures variability independent of absolute level and temporal trend in hemoglobin concentration. Greater hemoglobin variability is associated with diminished survival after adjusting for confounders and across multiple modeling strategies. Clinical trials comparing treatment strategies leading to differing degrees of hemoglobin variability will be needed to clarify the true causal nature of the associations

we have demonstrated. If hemoglobin variability were shown to have a causal association with mortality, it could become a novel target whose control among hemodialysis patients may reduce morbidity and prolong survival.

## CONCISE METHODS

### Study Population

Adult patients in the FMC database of hemodialysis patients were eligible for the study if they were alive during one of two enrollment periods: January 1, 1996, to June 30, 1996; or July 1, 1996, to December 31, 1996.

### Characterizing Hemoglobin Parameters

Hemoglobin variability, absolute hemoglobin level, and temporal hemoglobin trend were defined over study months 4 through 9. For each subject, a linear regression model of time on hemoglobin was fit, and Hb-Var was defined as the Res-SD of these regression models, which is essentially the SD of the differences between observed hemoglobin values and the regression line (Figure 1a). Absolute value of and temporal trends in hemoglobin levels were defined as the intercept and slope of the regression line, respectively. In addition, the average absolute change in hemoglobin was calculated over the same exposure windows. This was defined as the average of the absolute difference between consecutive hemoglobin measurements (Figure 1b).

### Outcomes

In the survival analyses, the outcome of interest was death from any cause. Patients were followed from enrollment until death or until a censoring event occurred. Censoring events included transfer from FMC facilities, transplantation, or end of study on September 30, 1998. Data on cause-specific death were not available, nor were hospitalization data.

### Covariates

Covariates were considered over study months 1 through 3. Demographic variables of interest included gender, race, and age at enrollment. Comorbid diseases of interest included diabetes (adult, juvenile, none) and duration of ESRD. Laboratory data included serum albumin, aspartate aminotransferase, bicarbonate, calcium, phosphate, parathyroid hormone, iron level, transferrin saturation (TSAT), ferritin, urea reduction ratio (URR), and Kt/V. [Because URR and Kt/V were collinear ( $r = 0.93$ ), multivariable models were fit with each variable separately, and again with both terms; effect estimates for parameters of interest were nearly identical in all cases.] Treatment-related variables of interest included intravenous iron dose and intravenous erythropoietin dose. Laboratory and treatment-related variables were updated at monthly intervals.

In models predicting Hb-Var, these data were considered as

candidate predictors. In survival analyses, these variables were considered as covariates.

### Statistical Analyses

The distribution of absolute level of hemoglobin, temporal trend, and Hb-Var (Res-SD) was described graphically and with summary statistics. Relationships between continuous variables were assessed using Pearson correlation coefficients and scatter plots. Potential predictors of Hb-Var were explored by fitting multivariable linear regression models; candidate predictors included all covariates mentioned above.

The associations between all-cause mortality and absolute level of hemoglobin, temporal trend in hemoglobin, and hemoglobin variability (Res-SD) were examined using adjusted Cox proportional hazards analysis. Hemoglobin parameters were measured over months 4 through 9 and used to predict mortality beginning immediately after month 9. We restricted observations to subjects who remained enrolled throughout the hemoglobin exposure window and had no missing hemoglobin values or covariate data during the exposure window.

We explored for potential nonlinear associations between our hemoglobin parameters and mortality in two ways. First, we utilized polynomial expressions (including quadratic and square root terms) for all hemoglobin parameters in our multivariable models. These higher-order terms were not significantly associated with outcome, nor did their inclusion improve overall model fit (data not shown). Second, we also fit our models with categorical forms of our three hemoglobin parameters: intercept, slope, and Res-SD. Using the lowest quartile for each hemoglobin parameter as the reference, the hazard ratios changed incrementally and monotonically across quartiles, further supporting the linear nature of the relationship between these parameters and mortality (data not shown). Thus we report findings based on linear descriptors.

We also explored interactions between Hb-Var and absolute level of hemoglobin, and between Hb-Var and temporal trend in hemoglobin using cross-product terms (*e.g.*, intercept  $\times$  Res-SD).

Standard regression methods may generate biased estimates of the causal association between hemoglobin variability and mortality in the presence of time-dependent confounding.<sup>34–36</sup> Time-dependent confounding occurs when variables such as iron or erythropoietin administration predict subsequent Res-SD and mortality and are associated with past levels of Res-SD (*i.e.*, they serve simultaneously as confounders and pathway intermediates).<sup>36</sup> To reduce the potential influence of time-dependent confounding, we repeated our primary analysis on subgroups of subjects with increasingly restrictive hemoglobin levels (*i.e.*, the minimum hemoglobin values during the exposure window were  $\geq 9.5$  g/dl,  $\geq 10$  g/dl,  $\geq 10.5$  g/dl). We postulated that subgroups defined by increasing minimum hemoglobin levels would have a lower prevalence of comorbid conditions that could function as time-dependent confounders.

All analyses were performed using SAS 9.1 (Cary, NC) (statistical code available upon request).

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## DISCLOSURES

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