

# Hemoglobin Variability Does Not Predict Mortality in European Hemodialysis Patients

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## ABSTRACT

Patients with CKD exhibit significant within-patient hemoglobin (Hb) level variability, especially with the use of erythropoiesis stimulating agents (ESAs) and iron. Analyses of dialysis cohorts in the United States produced conflicting results regarding the association of Hb variability with patient outcomes. Here, we determined Hb variability in 5037 European hemodialysis (HD) patients treated over 2 years to identify predictors of high variability and to evaluate its association with all-cause and cardiovascular disease (CVD) mortality. We assessed Hb variability with various methods using SD, residual SD, time-in-target (11.0 to 12.5 g/dl), fluctuation across thresholds, and area under the curve (AUC). Hb variability was significantly greater among incident patients than prevalent patients. Compared with previously described cohorts in the United States, residual SD was similar but fluctuations above target were less frequent. Using logistic regression, age, body mass index, CVD history, dialysis vintage, serum albumin, Hb, angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) use, ESA use, dialysis access type, dialysis access change, and hospitalizations were significant predictors of high variability. Multivariable adjusted Cox regression showed that SD, residual SD, time-in-target, and AUC did not predict all-cause or CVD mortality during a median follow-up of 12.4 months (IQR: 7.7 to 17.4). However, patients with consistently low levels of Hb (<11 g/dl) and those who fluctuated between the target range and <11 g/dl had increased risks for death (RR 2.34; 95% CI: 1.24 to 4.41 and RR 1.74; 95% CI: 1.00 to 3.04, respectively). In conclusion, although Hb variability is common in European HD patients, it does not independently predict mortality.

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The optimal target hemoglobin (Hb) concentration in patients with chronic kidney disease (CKD) remains a matter of considerable uncertainty. Randomized controlled trials have demonstrated that aiming for a “normal” Hb concentration does not improve outcomes but may increase the risk of cardiovascular morbidity and/or mortality.<sup>1–4</sup> These results suggest the optimal Hb may differ between the physiologic regulation of red cell formation and the therapeutic correction of anemia using erythropoiesis stimulating agents (ESAs) in CKD patients.

An apparent difference between CKD patients receiving ESAs and normal individuals is Hb vari-

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ability over time. A potential challenge in determining the clinical relevance of Hb fluctuations lies in the method used to assess Hb variability. Currently available metrics of variability mostly reflect a single aspect of variability (*e.g.*, magnitude, frequency, or duration)<sup>5,6</sup> and fail to simultaneously capture all components. Within-patient SD is the simplest measure of Hb variability, but it fails to discern patterns or directionality and cannot account for overall trends. Assessing variation derived from a regression line of Hb values (*i.e.*, “residual SD”) can account for overall trend but does not reflect patterns of variability.<sup>7</sup> The “time-in-target” is another easy-to-calculate measure, but as with SD and residual SD, it fails to account for variability patterns. Assessing Hb variability using the categories below, within or above a target range, has the advantage of describing the initial range of Hb measures, direction of change, and amplitude of change<sup>8,9</sup>; however, further statistical analysis becomes limited because it fails to provide a quantitative measure.

To date, large population-based studies on Hb variability have been based on hemodialysis (HD) patient populations in the United States.<sup>7–11</sup> Extrapolation of these data to European populations may not be adequate because of differences in patient characteristics and patterns of care, including higher ESA doses in the United States.<sup>12</sup>

The Analyzing data, Recognizing excellence, Optimizing outcomes (ARO) CKD research initiative aims to identify risk factors and opportunities for intervention in a large European dialysis cohort using a common database of patients from more than 150 Fresenius Medical Care dialysis centers in eastern and western Europe (EU-FME).<sup>13</sup> The aim of this study was to characterize Hb variability in these patients using different measures, to identify predictors of Hb variability, and to evaluate the association between Hb variability and mortality.

## RESULTS

### Patient Characteristics

Baseline characteristics of the 5037 patients included for analysis and those patients excluded (mainly because contiguous Hb measurements >6 months or other relevant data were not available) are shown in Supplemental Table S1. More than two-thirds of patients included in the analysis underwent dialysis treatment for >6 months (on dialysis for >6 months, herein referred to as “prevalent patients”). Less than half of patients excluded were prevalent, which presumably accounts for the differences in several characteristics between included and excluded patients. Patients included for analysis also had a lower mortality rate compared with those excluded (6 *versus* 27 deaths/100 population,  $P < 0.01$ ).

### Crossvalidation of Existing Measures of Hb Variability

Because of the limitations of using a single approach to determine Hb variability, we used a panel of different measures to

quantify variability over a 6-month period (herein referred to as “exposure period”): within-patient SD, residual SD, time-in-target, and the method of fluctuation across thresholds (target range 11.0 to 12.5 g/dl).<sup>5–9</sup> We also derived a novel approach to capture magnitude and frequency of variability as a single, quantitative index by integrating the area under the curve (AUC) between measured Hb values and the mean Hb concentration (Figure 1).

Measures of AUC were positively correlated with within-patient SD (Figure 2A;  $r = 0.85$ ,  $P < 0.01$ ) and residual SD (Figure 2B;  $r = 0.88$ ,  $P < 0.01$ ). AUC was negatively associated with time-in-target, but the correlation was weak ( $r = -0.17$ ,  $P < 0.01$ ) (Figure 2C). Results for AUC were consistent with the method of fluctuation across thresholds (Figure 3): patients who were consistently in target had the lowest median value of AUC (35 g/dl  $\times$  days), whereas patients in the high amplitude category had the largest median value for AUC (107 g/dl  $\times$  days) and the widest range (31 to 390 g/dl  $\times$  days).

Patients in the low-amplitude, high-Hb category were in target for  $85 \pm 50$  days; those in the low-amplitude, low-Hb category were in target for  $80 \pm 49$  days; and high-amplitude cyclers were in target for  $73 \pm 36$  days.

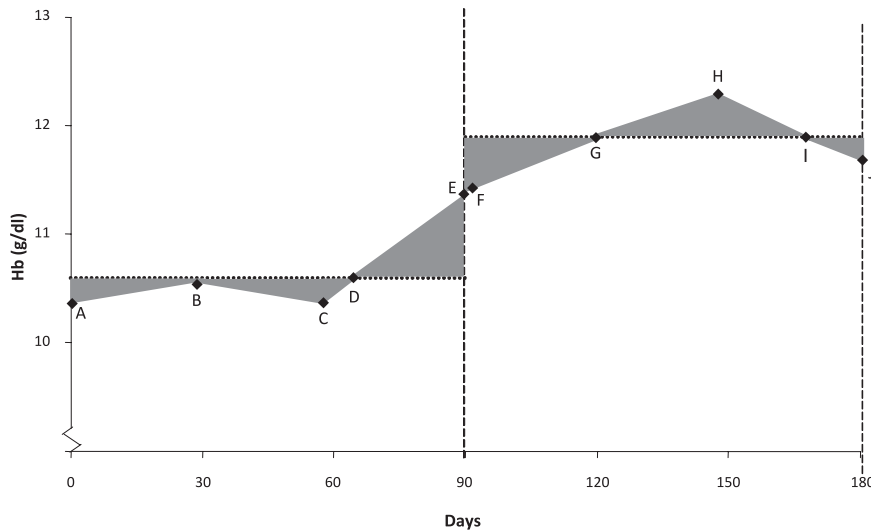
### Measures of Hb Variability in Incident and Prevalent Patients

Prevalent patients had a slightly higher mean Hb compared with patients who underwent dialysis therapy for <6 months (herein referred to as “incident patients”) and revealed a more stable Hb level during the exposure period (Table 1). Incident patients experienced a greater level of variability compared with prevalent patients (Table 1) as measured by within-patient SD, residual SD, and AUC. Incident patients spent less time-in-target and were more likely to be in the consistently low Hb category or in the high-amplitude Hb category.

### Predictors of Hb Variability

Patients in the highest AUC quartile were younger, thinner, and more often had diabetes compared with patients in the lower quartiles (Table 2). There were more incident patients in the higher quartiles of AUC, and they were less likely to have an arteriovenous fistula and more likely to have a change in vascular access type during the exposure period when Hb variability was assessed. Patients in the highest AUC quartile had a lower parathyroid hormone level and more frequently received cardiovascular disease (CVD)-related medication or an ESA. Moreover, they were 3 times more likely to have been hospitalized during the exposure period compared with those in the lowest quartile of AUC. The results for quartiles of SD were similar to those for AUC (data not shown).

Multivariable logistic regression showed that young age (<30 years), low body mass index, incident dialysis vintage, change in dialysis access, catheter use, anemia (Hb < 11 g/dl), ESA use, angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) use, and hospitalizations



**Figure 1.** Calculating AUC with the trapezoidal rule. To simultaneously capture the magnitude of variability and the frequency at which the fluctuations occur as a single, quantitative index, the AUC between measured Hb values and the mean Hb concentration was calculated. The letters A through J represent the points along a patient profile for Hb. We sliced Hb profiles into 90-day intervals in this study because (1) most Hb measures were taken monthly, (2) at least three measurements of Hb were needed to compute an AUC, and (3) 3 months provide ample time for patients to respond to ESA therapy and have a measurable effect on Hb. The summed integrations across the two 90-day intervals produce an overall index of variability in units of g/dl  $\times$  days. The area above the curve and the area below the curve were calculated separately for each 3-month interval. Integration was used to calculate the areas, approximating with the “trapezoidal rule”:

$$\sum \left( \frac{(y_i - m_j) + (y_{i+1} - m_j)}{2} \right) \cdot (d_{i+1} - d_i)$$

where  $y_i$  is the  $i^{\text{th}}$  Hb measurement taken on day  $d_i$  and  $m_j$  is the mean Hb value in quarter  $j$ . To integrate the areas, all points that make up the areas needed to be known, therefore it was necessary to linearly interpolate the days where the Hb profile intersected with the mean Hb reference line (points D, G, I) and the Hb value where the Hb profile intersected with the end of the interval (i.e., at day 90 and day 180 [points E and J]).

were important predictors of high Hb variability as measured by AUC (Table 3). A history of CVD and a higher serum albumin were negatively associated with high Hb variability.

### Hb Variability and Risk of Mortality

Patients were followed for a median of 12.4 months (interquartile range [IQR]: 7.7 to 17.4 months). Consequent to the open cohort design, prevalent patients had a longer duration of follow-up (16.0 months; IQR: 9.4 to 17.6 months) than incident patients (8.0 months; IQR: 4.0 to 12.1 months). Of the 551 deaths that occurred during this period, 220 (40%) were due to a CVD-related cause. Of these, 91 deaths were due to heart failure, 32 were due to myocardial infarction, and 45 were due to stroke. Overall, we found no statistically significant association between Hb variability and all-cause mortality (Table 4) as measured by SD, residual SD, time-in-target, and AUC. The only exceptions were observed in the crude model for quartile 2 of within-person SD and time-in-target <29 days; however, the relative risk estimates no

longer remained statistically significant with multivariable adjustment.

In contrast, results obtained using the method of fluctuations across thresholds showed a positive association between movement across the target range of Hb and mortality (Table 4). The crude analysis showed that patients who fell outside of the target range of 11 to 12.5 g/dl had a significantly greater risk of mortality (with the exception of patients in the low-amplitude high-Hb category). Results of Kaplan–Meier analysis (Figure 4) reinforce these findings. After multivariable adjustment, only patients in the consistently low Hb and the low-amplitude, low-Hb groups had a statistically significant increase in risk compared with patients who were consistently on target. When sensitivity analysis was performed using an 11- to 13-g/dl target range,<sup>14</sup> a similar pattern of relative risk was observed, although the magnitude was attenuated for all Hb categories; similar results were observed for a 10- to 12-g/dl target range (Supplemental Table S2).

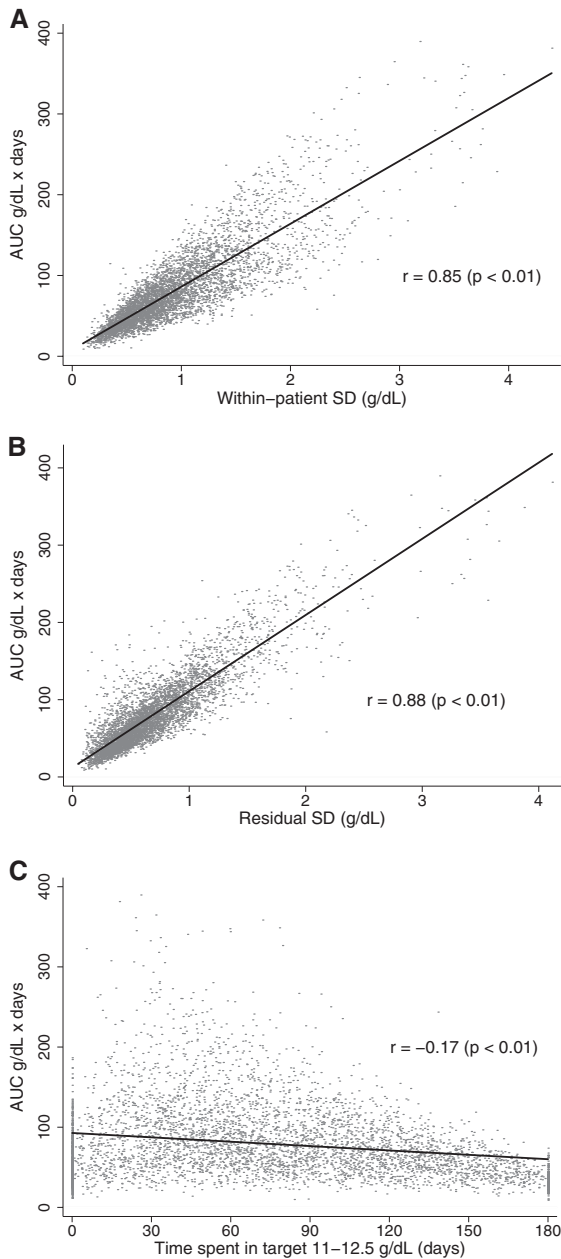
The overall results for CVD-related mortality were consistent with those for all-cause mortality (data not shown). A stratified analysis by dialysis vintage (incident *versus* prevalent) showed that Hb variability was not associated with an increased risk for all-cause mortality (data not shown). Results stratified by ESA use (ever use/never use) were consistent with those of the overall analysis (data not shown).

Results of sensitivity analysis using a 3- or 12-month period of Hb exposure (instead of 6-month) found no association between Hb variability and all-cause mortality (data not shown). Sensitivity analysis using the second 6 months of the study period as the exposure period (instead of the first 6 months) on the basis of AUC and SD and the method of fluctuations across target found no association between Hb variability and all-cause mortality. Sensitivity analysis in which the period of the first 3 months of Hb measurements was excluded for incident patients was also consistent with the overall results.

In contrast to Hb variability, low serum albumin at baseline was associated with increased mortality risk (Supplemental Table S3).

## DISCUSSION

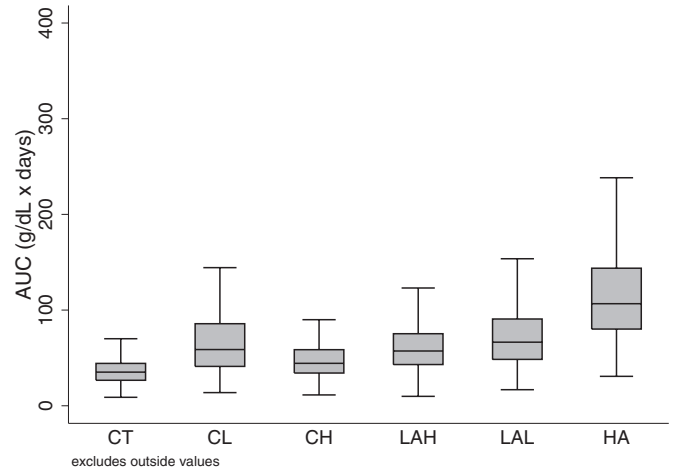
This study provides the first analysis of Hb variability and outcomes involving a large population of HD patients based outside the United States. It confirms that significant fluctuations



**Figure 2.** AUC is highly correlated with within-person SD (A) and residual SD (B) but not with time spent in target (C) ( $n = 5037$ ).

of Hb values occur in daily practice and provides novel insights into clinical factors that are potentially associated with greater Hb instability. In contrast to previous studies, our results do not confirm that Hb variability is an independent cause of mortality.

Various established measures were used for a comprehensive assessment of Hb variability. We also developed a new method, referred to as the “AUC approach,” to capture magnitude and frequency of the variability in a single quantitative index. An advantage of AUC is that it does not rely on assumptions with respect to the pattern of variability or the distribution of Hb values, whereas its limitations include the nondirectional-



**Figure 3.** Distribution of AUC is consistent with categories of method of fluctuation across thresholds. CT, consistently within the target range; CL, consistently low; CH, consistently high; LAH, low-amplitude fluctuation with high Hb levels; LAL, low-amplitude fluctuation with low Hb levels; HA, high-amplitude fluctuation. Frequency of patients in each group: CT = 228, CL = 376, CH = 292, LAH = 1145, LAL = 1682, and HA = 1314. Values above and below the whiskers have been excluded from the box plot. The whiskers are defined as the upper and lower adjacent values where the upper adjacent value is the largest data value that is less than or equal to the 75th percentile +  $1.5 \times$  IQR and the lower adjacent value is the smallest data value that is greater or equal to the 25th percentile -  $1.5 \times$  IQR.

ity. To validate AUC, we compared its results against those based on existing measures of variability. Results showed that AUC was highly correlated with within-patient SD and residual SD, which reinforces the utility of both measures of SD as a metric of Hb variability.

One interesting observation is that the instability of Hb values was larger among incident patients irrespective of the methodology used to measure Hb variability. This could be due to incident patients experiencing a slight increase in Hb levels during the exposure period, presumably because most of them did not receive ESAs before initiation of dialysis.<sup>15</sup> The higher level of variability may also be explained by a higher prevalence of comorbidities among incident patients.<sup>16</sup>

Our findings for residual SD were relatively consistent with previously reported findings. Although residual SD values of 0.65 and 0.77 g/dl in our study for prevalent and incident patients, respectively, are somewhat lower than values reported in the U.S. Medicare HD population (prevalent: 0.75 g/dl; incident: 0.95 g/dl),<sup>5</sup> a study in the prevalent North American Fresenius Medical Care population (FMC-NA) found a residual SD of 0.6 g/dl.<sup>7</sup> Despite the similarity of residual SD between the European and U.S. populations, the distribution of patients classified under the method of fluctuations across thresholds differs markedly. Three separate analyses of the prevalent U.S. Medicare population reported that <2% of patients were in the consistently low-Hb category; 5.9% to 6.5% in the consistently in-target category; <3% in the consistently

**Table 1.** Comparison of Hb variability measures by dialysis vintage

Hb Variability Measure	Total (n = 5037)	Prevalent (n = 3673)	Incident (n = 1364) <sup>a</sup>	P <sup>b</sup>
Mean Hb g/dl, mean (SD)	11.55 (1.23)	11.63 (1.22)	11.31 (1.22)	<0.01
Hb g/dl at the beginning of exposure period	11.40 (1.56)	11.63 (1.47)	10.77 (1.64)	<0.01
Hb g/dl at the end of exposure period	11.68 (1.46)	11.71 (1.46)	11.59 (1.44)	<0.01
SD g/dl, mean (SD)	0.92 (0.52)	0.86 (0.48)	1.08 (0.56)	<0.01
Residual SD g/dl, mean (SD)	0.68 (0.42)	0.65 (0.40)	0.77 (0.47)	<0.01
Time in target 11 to 12.5 g/dl (days), mean (SD)	73.1 (53.4)	75.5 (54.9)	66.7 (48.3)	<0.01
Fluctuations across thresholds, % (n)				
consistently within the target range (CT)	4.5 (228)	5.3 (194)	2.5 (34)	<0.01
consistently low (CL)	7.5 (376)	6.9 (253)	9.0 (123)	0.01
consistently high (CH)	5.8 (292)	6.9 (255)	2.7 (37)	<0.01
low amplitude with high Hb levels (LAH)	22.7 (1145)	24.9 (915)	16.9 (230)	<0.01
low amplitude with low Hb levels (LAL)	33.4 (1682)	32.9 (1208)	34.8 (474)	0.21
high amplitude (HA)	26.1 (1314)	23.1 (848)	34.2 (466)	<0.01
AUC (g/dl × days), mean (SD)	79.5 (47.2)	75.2 (45.0)	91.3 (50.9)	<0.01

<sup>a</sup>Incident dialysis was defined as <6 months of dialysis treatment.

<sup>b</sup>Comparing incident versus prevalent.

high category; 14.8% to 21.3% in the low-amplitude, low-Hb category; 28.9% to 36.4% in the low-amplitude, high-Hb category; and approximately 40% in the high-amplitude Hb category.<sup>5,8,9</sup> Prevalent patients in our study were more frequently found in the consistently low, consistently high, and low-amplitude, low-Hb categories (6.9%, 6.9%, and 32.9%, respectively) and less frequently in the low-amplitude, high-Hb and high-amplitude categories (24.9% and 23.1%, respectively), indicating less frequent fluctuations into the above target range and thus possibly a more conservative approach to raising Hb levels in Europe.

Various parameters known to be associated with severity of renal anemia and/or higher ESA dose requirements are being considered as possible causes of Hb variability.<sup>6</sup> Interestingly, the analysis presented here could only confirm a role for some of these factors and the mechanisms by which some predictors affect Hb stability are not immediately apparent. Hospitalizations, change in vascular access, and not having an arteriovenous fistula were associated with greater variability and could affect blood loss, erythropoietic responsiveness, or ESA dosing. A significant association between hospitalizations and Hb variability was also observed in other studies.<sup>17</sup> The observation that ESA use itself is a predictor of Hb variability could be because patients not receiving ESA can still adjust their residual endogenous erythropoietin formation. It could also indicate that the pharmacokinetics and pharmacodynamics of ESAs and dosing adjustments contribute to greater Hb variability. Recently it was reported that among nondialysis CKD patients, Hb variability is increased in ESA users.<sup>18</sup>

As in a previous study, higher serum albumin levels were inversely associated with Hb variability,<sup>17</sup> which is consistent with low serum albumin being a surrogate for comorbidity and inflammation. In contrast, C-reactive protein values were identical across quartiles of AUC and SD. Why lower age and body mass index predicted greater Hb variability is less clear, but a similar association with age has been reported previously.<sup>17</sup> Equally unclear is the association between the absence of

CVD history or antihypertensive medication use and Hb variability.

It is important to note that no consistent algorithm for dose adjustments of ESAs was used in the participating facilities. However, recommended target ranges were different, ranging from between 9 and 12 g/dl (Slovak Republic) to between 11 and 13 g/dl (Czech Republic). Adjustment for these different Hb target ranges as part of sensitivity analysis did not modify the study conclusions (data not shown).

Irrespective of the complexity of the underlying mechanisms, we were unable to confirm that Hb variability is an independent risk factor for all-cause or CVD mortality. In contrast, Yang *et al.*<sup>7</sup> previously found an association of Hb variability and mortality in a large FMC-NA cohort prevalent in 1996. In this cohort, the magnitude of this association became larger when the analysis was restricted to subgroups of Hb to address time-dependent confounding.<sup>19</sup> However, an analysis of a smaller, more recent incident cohort by the same investigators found that Hb variability was not associated with decreased survival.<sup>10</sup> The investigators concluded that there may be important differences between incident and prevalent cohorts with respect to the effect of Hb variability or perhaps that changes in anemia management over time might account for the different role of Hb variability. The results of this study were stratified by dialysis vintage; however, we found no association between Hb variability and mortality in either patient group.

Although Hb variability *per se* was not associated with decreased survival, patients in the consistently low-Hb category and those in the low-amplitude, low-Hb category had an increased risk for mortality. These observations are consistent with a recent analysis by Gilbertson *et al.*,<sup>9</sup> who found the number and timing of Hb values <11 g/dl and falling Hb values were associated with increased mortality. The cause and effect of this relationship cannot easily be established given the absence of randomized controlled trials comparing the currently recommended Hb target range with a lower range.<sup>20</sup> On the other hand, it appears noteworthy in light of the concern about the risks associated with

**Table 2.** Patient characteristics by quartile of AUC

Patient Characteristics	<47.0 g/dl × days (n = 1259)	47.0 to <68.2 g/dl × days (n = 1259)	68.2 to <99.1 g/dl × days (n = 1259)	≥99.1 g/dl × days (n = 1260)	Total (n = 5037)
Age (years)	62.7 ± 13.7	62.6 ± 14.3	62.2 ± 14.4	60.8 ± 15.9	62.1 ± 14.6
Gender					
female	535 (42.5)	544 (43.2)	584 (46.4)	552 (43.8)	2215 (44.0)
male	724 (57.5)	715 (56.8)	675 (53.6)	708 (56.2)	2822 (56.0)
Body mass index (kg/m <sup>2</sup> )	26.3 ± 4.9	26.0 ± 4.9	25.5 ± 4.9	24.6 ± 4.6	25.6 ± 4.8
Smoking status					
nonsmoker	815 (64.7)	793 (63.0)	785 (62.4)	779 (61.8)	3172 (63.0)
former/current	307 (24.4)	318 (25.3)	320 (25.4)	335 (26.6)	1280 (25.4)
missing data	137 (10.9)	148 (11.8)	154 (12.2)	146 (11.6)	585 (11.6)
History of diabetes	291 (23.1)	303 (24.1)	334 (26.5)	334 (26.5)	1262 (25.1)
History of CVD	1051 (83.5)	1028 (81.7)	1002 (79.6)	928 (73.7)	4009 (79.6)
History of cancer	78 (6.2)	60 (4.8)	69 (5.5)	62 (4.9)	269 (5.3)
CKD etiology					
hypertension/vascular	163 (12.9)	192 (15.3)	177 (14.1)	185 (14.7)	717 (14.2)
glomerulonephritis	202 (16.0)	229 (18.2)	214 (17.0)	216 (17.1)	861 (17.1)
diabetic nephropathy	167 (13.3)	162 (12.9)	195 (15.5)	190 (15.1)	714 (14.2)
tubulointerstitial	178 (14.1)	179 (14.2)	191 (15.2)	191 (15.2)	739 (14.7)
polycystic kidney disease	96 (7.6)	77 (6.1)	78 (6.2)	65 (5.2)	316 (6.3)
miscellaneous	40 (3.2)	37 (2.9)	30 (2.4)	49 (3.9)	156 (3.1)
unknown	413 (32.8)	383 (30.4)	374 (29.7)	364 (28.9)	1534 (30.5)
Dialysis vintage					
prevalent	1046 (83.1)	957 (76.0)	879 (69.8)	791 (62.8)	3673 (72.9)
incident	213 (16.9)	302 (24.0)	380 (30.2)	469 (37.2)	1364 (27.1)
Vascular access type					
fistula	1067 (84.7)	1013 (80.5)	986 (78.3)	940 (74.6)	4006 (79.5)
graft	39 (3.1)	48 (3.8)	54 (4.3)	45 (3.6)	186 (3.7)
catheter (temporary)	36 (2.9)	46 (3.7)	56 (4.4)	86 (6.8)	224 (4.4)
catheter (permanent)	62 (4.9)	89 (7.1)	117 (9.3)	130 (10.3)	398 (7.9)
other/missing	55 (4.4)	63 (5.0)	46 (3.7)	59 (4.7)	223 (4.4)
Vascular access type changed at least once	57 (4.5)	108 (8.6)	145 (11.5)	207 (16.4)	517 (10.3)
Dialysis parameters					
Kt/V	1.6 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.6 ± 0.3	1.6 ± 0.3
actual blood flow (ml/min)	318.6 ± 44.6	310.7 ± 49.3	308.8 ± 47.7	306.5 ± 49.4	311.2 ± 48.0
Laboratory parameters					
C-reactive protein (mg/L)	6.5 (3.0, 12.4)	7.1 (3.1, 14.8)	7.5 (3.3, 15.6)	6.7 (3.1, 13.6)	7.0 (3.1, 14.0)
serum albumin (g/dl)	4.0 ± 0.4	4.0 ± 0.4	3.9 ± 0.5	3.9 ± 0.5	3.9 ± 0.4
cholesterol (mmol/L)	4.5 ± 1.1	4.5 ± 1.1	4.4 ± 1.1	4.3 ± 1.1	4.4 ± 1.1
calcium (mg/dl)	9.2 ± 0.8	9.2 ± 0.9	9.1 ± 0.8	9.1 ± 0.8	9.1 ± 0.8
phosphate (mg/dl)	4.8 ± 1.1	4.7 ± 1.2	4.7 ± 1.2	4.8 ± 1.2	4.8 ± 1.2
parathyroid hormone (pg/ml)	207 (106, 386)	209 (106, 379)	190 (93, 355)	186 (94, 341)	198 (100, 369)
Hb (g/dl)	11.8 ± 1.2	11.7 ± 1.2	11.4 ± 1.2	11.3 ± 1.2	11.5 ± 1.2
ferritin (μg/L)	458 (264, 739)	455 (262, 750)	455 (252, 772)	457 (246, 794)	456 (255, 766)
leukocytes (number/mm <sup>3</sup> )	6760 ± 2016	6715 ± 1873	6935 ± 2031	6847 ± 2229	6815 ± 2044
Medication					
ACE inhibitor or ARB use	177 (14.1)	245 (19.5)	271 (21.5)	314 (24.9)	1007 (20.0)
other antihypertensive agents	282 (22.4)	343 (27.2)	356 (28.3)	429 (34.0)	1410 (28.0)
oral anticoagulants	29 (2.3)	41 (3.3)	38 (3.0)	40 (3.2)	148 (2.9)
anti-aggregants	213 (16.9)	250 (19.9)	269 (21.4)	292 (23.2)	1024 (20.3)
oral vitamin D	293 (23.3)	324 (25.7)	326 (25.9)	335 (26.6)	1278 (25.4)
phosphate binders	608 (48.3)	633 (50.3)	660 (52.4)	676 (53.7)	2577 (51.2)
cinacalcet	7 (0.6)	12 (1.0)	9 (0.7)	14 (1.1)	42 (0.8)
ESA	885 (70.3)	981 (77.9)	1069 (84.9)	1135 (90.1)	4070 (80.8)
Hospitalized at least once	79 (6.3)	122 (9.7)	189 (15.0)	245 (19.4)	635 (12.6)

Vascular access information, dialysis parameters, laboratory measurements, medications, and hospitalizations were measured during the 6-month exposure period; all other variables were recorded at baseline. Mean ± SD is reported if the variable is normally distributed; median (IQR) is reported otherwise. Categorical variables are reported using n (%). Incident dialysis was defined as <6 months of dialysis treatment. ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

high-Hb targets that in the study presented here and in previous analyses<sup>9,21</sup> those patients who had achieved Hb levels consistently >12.5 g/dl did not show an increase in mortality risk. When different Hb ranges (11 to 13 g/dl or 10 to 12 g/dl) than 11 to 12.5 g/dl were used to analyze patient risks, the relative risk in the consistently low Hb category remained elevated; however, the relative

risk for all other Hb categories (including consistently above target) remained near 1.0.

The limitations of this study include the observational nature, with the inherent difficulties to determine cause-and-effect relationships, and the fact that the results are based on a clinical database that was not originally estab-

**Table 3.** Predictors of Hb variability (AUC): odds ratio of developing "high" variability (defined as > median Hb variability)

Variable	Level	Odds Ratio	Lower 95% CI	Upper 95% CI
Age	<30 years (reference)	1.00	—	—
	30 to 39 years	0.51	0.32	0.82
	40 to 49 years	0.47	0.30	0.72
	50 to 59 years	0.46	0.30	0.70
	60 to 69 years	0.43	0.28	0.66
	70 to 79 years	0.41	0.27	0.63
	80 to 89 years	0.41	0.26	0.65
	≥90 years	0.36	0.16	0.83
Gender	Female	1.06	0.91	1.22
	Male (reference)	1.00	—	—
Body mass index	<18 kg/m <sup>2</sup>	1.21	0.74	1.99
	18 to <25 kg/m <sup>2</sup> (reference)	1.00	—	—
	25 to <30 kg/m <sup>2</sup>	0.86	0.74	1.01
	30 to <35 kg/m <sup>2</sup>	0.62	0.50	0.78
	≥35 kg/m <sup>2</sup>	0.50	0.35	0.70
	Missing	0.84	0.67	1.05
Smoking	None	1.00	—	—
	Former	1.03	0.87	1.23
	Current	1.07	0.83	1.37
	Missing	1.29	1.05	1.59
CVD history	Present versus absent	0.82	0.69	0.98
Diabetes history	Present versus absent	1.06	0.85	1.30
Cancer history	Present versus absent	0.92	0.71	1.20
CKD etiology	Glomerulonephritis (reference)	1.00	—	—
	Hypertension/vascular	1.07	0.85	1.34
	Diabetic nephropathy	1.07	0.81	1.41
	Tubulointerstitial nephritis	1.13	0.91	1.41
	Polycystic kidney disease	1.10	0.82	1.47
	Miscellaneous	1.06	0.73	1.53
	Unknown	1.07	0.87	1.31
	Missing	0.74	0.56	0.97
Vintage	Incident versus prevalent	1.48	1.26	1.75
Dialysis access	AV fistula (reference)	1.00	—	—
	Graft	1.28	0.91	1.80
	Temporary catheter (vascular)	1.63	1.21	2.21
	Permanent catheter (vascular)	1.54	1.21	1.95
	Other/Missing	1.07	0.74	1.55
Leucocytes <sup>a</sup>	Q1 (reference)	1.00	—	—
	Q2	0.87	0.72	1.05
	Q3	0.84	0.69	1.01
	Q4	1.03	0.85	1.25
	Missing	0.61	0.29	1.28
Blood flow <sup>a</sup>	Q1 (reference)	1.00	—	—
	Q2	0.86	0.71	1.03
	Q3	0.76	0.62	0.92
	Q4	0.88	0.71	1.10
	Missing	1.13	0.82	1.55
KtV/sp <sup>a</sup>	Q1 (reference)	1.00	—	—
	Q2	1.08	0.91	1.29
	Q3	1.09	0.91	1.32
	Q4	1.05	0.85	1.30
	Missing	0.76	0.47	1.22
C-reactive protein <sup>a</sup>	Q1 (reference)	1.00	—	—
	Q2	1.08	0.90	1.30
	Q3	1.11	0.92	1.35
	Q4	1.04	0.86	1.26
	Missing	0.91	0.74	1.12

Table 3. Continued

Variable	Level	Odds Ratio	Lower 95% CI	Upper 95% CI
Albumin <sup>a</sup>	Q1 (reference)	1.00	—	—
	Q2	0.88	0.73	1.05
	Q3	0.87	0.73	1.05
	Q4	0.77	0.63	0.93
	Missing	0.92	0.65	1.30
Calcium <sup>a</sup>	<8.4 mg/dl	1.13	0.94	1.37
	8.4 to 9.5 mg/dl (reference)	1.00	—	—
	>9.5 to 11 mg/dl	0.89	0.77	1.03
	>11 mg/dl	0.90	0.54	1.50
	Missing	1.42	0.75	2.66
Phosphate <sup>a</sup>	<3.5 mg/dl	0.85	0.71	1.02
	3.5 to 5.5 mg/dl (reference)	1.00	—	—
	>5.5 mg/dl	1.14	0.98	1.33
	Missing	0.71	0.45	1.12
Ferritin <sup>a</sup>	≤100 μg/L (reference)	1.00	—	—
	>100 to 500 μg/L	0.94	0.74	1.20
	>500 μg/L	1.07	0.83	1.38
	Missing	1.54	0.96	2.48
Cholesterol <sup>a</sup>	Q1 (reference)	1.00	—	—
	Q2	1.09	0.92	1.30
	Q3	0.97	0.81	1.15
	Q4	0.96	0.80	1.15
	Missing	1.13	0.78	1.63
Parathyroid hormone <sup>a</sup>	<75 pg/ml	1.14	0.94	1.39
	75 to <150 pg/ml	1.03	0.86	1.24
	150 to 300 pg/ml (reference)	1.00	—	—
	>300 to 600 pg/ml	0.84	0.69	1.01
	>600 to 800 pg/ml	1.13	0.82	1.56
	>800 pg/ml	0.83	0.62	1.10
	Missing	0.85	0.68	1.08
Hb <sup>a</sup>	<11 g/dl	1.37	1.17	1.61
	11 to 12 g/dl (reference)	1.00	—	—
	>12 to 13 g/dl	0.94	0.80	1.11
	>13 g/dl	0.89	0.71	1.12
ACE inhibitor or ARB use <sup>a</sup>	Yes versus no	1.34	1.13	1.58
ESA <sup>a</sup>	Yes versus no	2.03	1.71	2.41
Phosphate binder <sup>a</sup>	None (reference)	1.00	—	—
	Calcium-containing only	0.96	0.82	1.12
	Other	0.93	0.78	1.11
Antihypertensive <sup>a</sup>	Yes versus no	1.05	0.91	1.22
Oral anticoagulants <sup>a</sup>	Yes versus no	0.95	0.66	1.36
Anti-aggregants <sup>a</sup>	Yes versus no	1.03	0.88	1.21
Vitamin D (oral) <sup>a</sup>	Yes versus no	1.05	0.90	1.22
Dialysis access change <sup>a</sup>	Yes versus no	1.44	1.16	1.80
Hospitalization <sup>a</sup>	Yes versus no	2.03	1.66	2.48

Predictors of high variability (defined as AUC > 50th percentile: 68.2 g/dl × days) were evaluated using baseline variables (age, gender, country, body mass index, smoking status, history of diabetes, history of CVD, history of cancer, CKD etiology, dialysis vintage) and variables measured during the 6-month Hb evaluation period (CRP, serum albumin, calcium, phosphate, parathyroid hormone, Hb, ferritin, cholesterol, blood leukocyte count, oral vitamin D, phosphate binders, ACE inhibitors and ARBs, other antihypertensive drugs, oral anticoagulants, anti-aggregants, Kt/V, vascular access type, actual blood flow, ESA use, change in vascular access type, and hospitalizations). Logistic regression was used to show the effect of the predictors in the full model. Q, quartile.

<sup>a</sup>Measured during the course of 6-month exposure period.

lished as a research tool.<sup>22</sup> A more specific limitation is that the database is from a single private dialysis provider (EU-FME). Thus, the generalizability to patients under the care of other providers and in countries without EU-FME centers remains unclear. Another limitation is the large percentage of patients (44%) who were excluded from analysis

as a result of the open cohort design (52% of excluded patients were incident), which may have resulted in selection bias. The absence of information on iron administration is another limitation because iron therapy may have influenced Hb variability or patient survival. Furthermore, the median observation period was approximately 1 year, which

**Table 4.** Relative risk of mortality by category of Hb variability

Hb Variability Measure	Category	Crude Hazard Ratio (95% CI)	Adjusted Hazard Ratio (95% CI) <sup>a</sup>	Number at Risk	Number of Events
Within-patient SD	<0.55 g/dl	1.00	1.00	1259	126
	0.55 to <0.79 g/dl	1.34 (1.06 to 1.69)	1.24 (0.97 to 1.57)	1259	158
	0.79 to <1.16 g/dl	1.16 (0.91 to 1.48)	1.02 (0.79 to 1.31)	1258	135
	≥1.16 g/dl	1.23 (0.96 to 1.57)	1.12 (0.86 to 1.45)	1261	132
Residual SD	<0.40 g/dl	1.00	1.00	1259	127
	0.40 to <0.58 g/dl	1.25 (0.99 to 1.59)	1.25 (0.98 to 1.60)	1259	153
	0.58 to <0.85 g/dl	1.21 (0.95 to 1.54)	1.07 (0.84 to 1.38)	1259	142
	≥0.85 g/dl	1.10 (0.86 to 1.41)	0.99 (0.76 to 1.30)	1260	129
Time-in-target 11 to 12.5 g/dl <sup>b</sup>	114 to 180 days	1.00	1.00	1260	119
	68 to <114 days	1.19 (0.93 to 1.53)	1.23 (0.95 to 1.60)	1262	135
	29 to <68 days	1.25 (0.98 to 1.60)	1.26 (0.95 to 1.68)	1256	139
	<29 days	1.40 (1.11 to 1.78)	1.36 (0.98 to 1.89)	1259	158
Fluctuations across thresholds	Consistently within the target range (CT)	1.00	1.00	228	15
	Consistently low (CL)	2.85 (1.62 to 5.02)	2.34 (1.24 to 4.41)	376	60
	Consistently high (CH)	1.98 (1.08 to 3.63)	1.79 (0.84 to 3.82)	292	35
	Low amplitude with high Hb levels (LAH)	1.57 (0.92 to 2.70)	1.47 (0.81 to 2.69)	1145	110
	Low amplitude with low Hb levels (LAL)	1.95 (1.15 to 3.30)	1.74 (1.00 to 3.04)	1682	198
AUC	High amplitude (HA)	1.85 (1.09 to 3.16)	1.57 (0.91 to 2.71)	1314	133
	<0.47 g/dl × days	1.00	1.00	1259	130
	0.47 to <68.2 g/dl × days	1.13 (0.89 to 1.43)	1.05 (0.82 to 1.34)	1259	141
	68.2 to <99.1 g/dl × days	1.25 (0.99 to 1.58)	1.03 (0.81 to 1.33)	1259	150
	≥99.1 g/dl × days	1.13 (0.88 to 1.44)	1.00 (0.77 to 1.31)	1260	130

Categories of variability for all measures (with the exception of fluctuation across thresholds) were defined using quartiles.

<sup>a</sup>Adjusted for demographics (age, gender, country, BMI, smoking status), medical history (diabetes, CVD, cancer, CKD etiology), dialysis parameters (dialysis vintage, Kt/V, vascular access type, actual blood flow), markers of inflammation (CRP, serum albumin), CVD medication (ACE inhibitor or ARB use, other antihypertensives, oral anticoagulants, anti-aggregants), bone and mineral metabolism medication (cinacalcet, oral vitamin D, phosphate binders), other lab parameters (calcium, phosphate, parathyroid hormone, Hb, ferritin, cholesterol, blood leukocyte count), ESA use, change in vascular access type, hospitalization). Adjustment for country-specific Hb target values rather than countries revealed similar results (data not shown).

<sup>b</sup>11- to 12.5-g/dl range is consistent with Ebben *et al.*<sup>8</sup> based on analysis of U.S. Renal Data System.

precludes any conclusions on longer-term mortality. Although the study included >5000 patients, the numbers of events in subgroups of patients were relatively small. Nev-

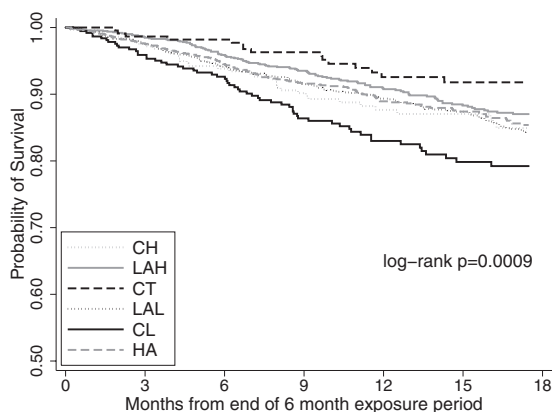
ertheless, study characteristics were sufficient to confirm that low serum albumin, an established risk marker,<sup>23</sup> predicts poor outcomes.

In conclusion, Hb variability occurs in the European HD population to a similar extent as in U.S. HD populations. It is related to various patient characteristics, comorbidities, and hospitalizations but is not an independent risk factor for all-cause or CVD mortality.

## CONCISE METHODS

### Study Population

The entire population consisted of randomly selected HD patients treated between January 2005 and December 2006 at an EU-FME facility in one of 11 countries: Czech Republic, France, Hungary, Italy, Poland, Portugal, Slovak Republic, Slovenia, Spain, Turkey, and the United Kingdom ( $n = 11,153$ ).<sup>13</sup> We excluded patients from centers where most data on actual blood flow or Kt/V were missing ( $n = 1352$ ). U.K. patients were excluded because of missing information on medications ( $n = 838$ ). From the remaining 8963 patients, 5427 patients were selected who had at least 6 months of contiguous monthly



**Figure 4.** Kaplan–Meier analysis showing patients with consistently low hemoglobin levels have the highest risk of mortality ( $n = 5037$ ). CT, consistently within the target range; CL, consistently low; CH, consistently high; LAH, low-amplitude fluctuation with high Hb levels; LAL, low-amplitude fluctuation with low Hb levels; HA, high-amplitude fluctuation.

Hb measurements over the exposure period. Patients were excluded if they had a bleeding episode or a blood transfusion ( $n = 390$ ) during follow-up, resulting in a study cohort of 5037 patients. Patients were classified as incident if they had received HD therapy for  $<6$  months at the time of enrollment.

### Assessment of Hb Variability

Hb variability was assessed using within-patient SD, residual SD, time-in-target, and the method of fluctuation across thresholds over a 6-month period of evaluation.<sup>7–9</sup> An 11.0- to 12.5-g/dl target was used for time-in-target and the method of fluctuation across thresholds. Sensitivity analysis was performed using 10- to 12-g/dl and 11- to 13-g/dl targets. We also derived a novel approach to capture the magnitude of variability and the frequency at which the fluctuations occur as a quantitative index referred to as the AUC approach (Figure 1).

### Statistical Analysis

Unadjusted comparisons were performed using a paired or independent  $t$  test,  $\chi^2$  test, or Wilcoxon rank-sum test as appropriate. Poisson regression was used to assess mortality rates. Predictors of high variability (defined as AUC  $>$  50th percentile: 68.2 g/dl  $\times$  days) were evaluated using logistic regression.

Cox regression was used to examine the association between Hb variability and mortality. Subjects began to accrue risk for up to 18 months after the Hb variability was assessed (*i.e.*, the exposure period) until death or a censoring event. Patients were considered lost to follow-up if they left a dialysis facility and did not return within 45 days. Sensitivity analysis evaluated the effect of changing the duration of Hb exposure (3 and 12 months). We reanalyzed the data using the second 6 months of Hb variability measurements. Furthermore, we evaluated the effect of excluding the first 3 months of incident patients' follow-up. Time-to-event analysis was performed using Kaplan–Meier methods to evaluate survival to 18 months.

All statistical analyses were performed using Stata (version 10.0, College Station, TX) and were reproduced independently by a second statistician using SAS (version 9.0, Cary, NC).

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### DISCLOSURES

Kai-Uwe Eckardt has received consulting or lecture fees from Affymax, Amgen, Johnson & Johnson, Kirin, Roche, and Sandoz Hexal. Florian Kronenberg

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