

Carbamylation of Serum Albumin and Erythropoietin Resistance in End Stage Kidney Disease

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Summary

Background and objectives The mechanisms underlying erythropoietin resistance are not fully understood. Carbamylation is a post-translational protein modification that can alter the function of proteins, such as erythropoietin. The hypothesis of this study is that carbamylation burden is independently associated with erythropoietin resistance.

Design, setting, participants, & measurements In a nonconcurrent prospective cohort study of incident hemodialysis patients in the United States, carbamylated albumin, a surrogate of overall carbamylation burden, in 158 individuals at day 90 of dialysis initiation and erythropoietin resistance index (defined as average weekly erythropoietin dose [U] per kg body weight per hemoglobin [g/dl]) over the subsequent 90 days were measured. Linear regression was used to describe the relationship between carbamylated albumin and erythropoietin resistance index. Logistic regression characterized the relationship between erythropoietin resistance index, 1-year mortality, and carbamylation.

Results The median percent carbamylated albumin was 0.77% (interquartile range=0.58%–0.93%). Median erythropoietin resistance index was 18.7 units/kg per gram per deciliter (interquartile range=8.1–35.6 units/kg per gram per deciliter). Multivariable adjusted analysis showed that the highest quartile of carbamylated albumin was associated with a 72% higher erythropoietin resistance index compared with the lowest carbamylation quartile ($P=0.01$). Increasing erythropoietin resistance index was associated with a higher risk of death (odds ratio per unit increase in log-erythropoietin resistance index, 1.69; 95% confidence interval, 1.06 to 2.70). However, the association between erythropoietin resistance index and mortality was no longer statistically significant when carbamylation was included in the analysis (odds ratio, 1.44; 95% confidence interval, 0.87 to 2.37), with carbamylation showing the dominant association with death (odds ratio for high versus low carbamylation quartile, 4.53; 95% confidence interval, 1.20 to 17.10).

Conclusion Carbamylation was associated with higher erythropoietin resistance index in incident dialysis patients and a better predictor of mortality than erythropoietin resistance index.

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Introduction

The kidney's production of erythropoietin is impaired in CKD, and erythropoiesis-stimulating agents (ESAs), such as recombinant human erythropoietin (EPO), are commonly used to manage the anemia of CKD. Although most patients experience a dose-dependent response to ESAs, some individuals are considered resistant, showing a limited or absent response despite high-dose treatment (1). The mechanisms of EPO resistance remain poorly understood but are likely multifactorial, relating to iron stores, inflammation, and other causes (1). Although lower EPO responsiveness is associated with an increased risk of adverse cardiovascular events, including death (2–4), it is unclear if these observed risks are caused by ESAs themselves or underlying processes leading to higher EPO requirements. Furthermore, it remains clinically challenging to accurately predict which patients will

be resistant to EPO and how such a course can be altered.

Carbamylation is the nonenzymatic binding of urea-derived cyanate to free amino groups on proteins, and it accumulates as kidney function declines (5,6). The post-translational protein alterations of carbamylation have been implicated in the progression of various diseases by changing the charge, structure, and function of enzymes, hormones, and receptors (7,8). For example, when carbamylated, proteins as diverse as collagen and LDL accelerate the biochemical events of atherosclerosis (8,9), and carbamylation of EPO abrogates its erythropoietic activity both *in vitro* and *in vivo* (10–12). Proteins with long half-lives provide a time-averaged indication of carbamylation burden analogous to the relationship between serum glucose and glycated hemoglobin (5,7,13). Markers of total carbamylation burden, such as homocitrulline

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and carbamylated albumin, are associated with increased risk of cardiovascular events and death in both dialysis and nondialysis populations (14–16). Because carbamylation seems to be a modifiable process, it serves as a potential therapeutic target aimed at altering its pathophysiological consequences (14,15,17).

The proportion of carbamylated albumin is strongly correlated with blood urea concentrations and strongly associated with all-cause mortality in hemodialysis patients (15). The disease mechanisms connecting protein carbamylation to mortality, however, remain unclear. Herein, we characterized the relationship between albumin carbamylation and EPO responsiveness in incident hemodialysis patients, hypothesizing that carbamylation burden is independently associated with EPO resistance.

Materials and Methods

Study Population

The Accelerated Mortality on Renal Replacement (ArMORR) study is a prospective cohort study of 10,044 incident hemodialysis patients in any of 1056 US centers operated by Fresenius Medical Care North America (FMC) between June of 2004 and August of 2005 (18–20). All participants underwent 1 year of follow-up, except for those participants who died (15.2%), voluntarily discontinued dialysis (5%), underwent kidney transplantation (3%), recovered renal function (4%), or transferred to a dialysis unit outside the FMC system before completing 1 year of hemodialysis (12%). Clinical data were prospectively collected by physicians at the point of care and included demographic information, coexisting conditions, results of studies performed by a central laboratory (Spectra East, Northvale, NJ), medication administration, and outcomes. Plasma and serum samples that would otherwise have been discarded after routine clinical testing were saved and stored in liquid nitrogen. The study was approved by the Institutional Review Board of the Massachusetts General Hospital, which waived the need for informed consent from each patient, because all personal identifiers were removed from the blood samples and clinical data before transfer to the investigators.

We previously evaluated the association between carbamylated albumin and mortality in the ArMORR cohort through a nested case-control study of 81 randomly selected patients who died within the first year of dialysis (cases) and 106 patients who were alive 1 year after starting dialysis (controls) frequency-matched for sex, race, age, and cause of ESRD (15). All individuals were receiving EPO therapy throughout the study period, and we have shown that this subset of patients had similar characteristics to others who died or survived, respectively, in the larger ArMORR cohort (15). Thereafter, we excluded 29 patients who did not have complete data to measure EPO responsiveness over the 90-day follow-up period (died within that vintage, final $n=158$).

Exposure and Outcome

Our primary exposure was carbamylated albumin as a percent of total (%C-Alb) measured 90 days after starting hemodialysis to reflect the burden of carbamylation accumulation subsequent to transitioning to maintenance

hemodialysis. Carbamylated albumin was measured by HPLC and tandem mass spectrometry as previously described (coefficient of variation of 4.2%) (15). The primary outcome was EPO resistance index (ERI) (21), which was calculated as the average weekly weight-adjusted dose of EPO (U/kg per week) divided by the average hemoglobin concentration (g/dl) measured between days 90 and 180. Because protein carbamylation may contribute directly to the causes of EPO resistance, we determined if baseline %C-Alb values (day 90) were predictive of subjects' ERI during the ensuing several months (days 90–180). Moreover, ERI calculations using averaged measures were used to reduce single measure variability (21–23).

Statistical Methods

Baseline characteristics are summarized for the overall population (primary analysis) and then by mortality status (secondary analysis). Demographic data were recorded at the start of dialysis initiation, whereas baseline laboratory values and baseline EPO dose represent averages over the first 90 days of dialysis (before the carbamylation measure). Using counts, differences by mortality status for categorical

Table 1. Baseline characteristics for the study population (primary analysis)

Variable	All Subjects ($n=158$)
Age, yr	70±12.8
Women	81 (51.3)
White race	106 (67.1)
Comorbidities	
Coronary artery disease	20 (12.7)
Congestive heart failure	33 (20.9)
Diabetes mellitus ^a	43 (27.2)
Hypertensive renal disease	62 (52.9)
Glomerulonephropathy	33 (20.9)
Vascular access: catheter	93 (61.6)
Body mass index (kg/m ²)	26.9±7.2
Systolic BP (mmHg)	144.9±24.2
Diastolic BP (mmHg)	72.3±12.7
Urea reduction ratio	70.2±7.8
Laboratory data	
BUN (mg/dl)	46 (38–57)
Hemoglobin (g/dl)	10.5 (9.6–11.3)
Albumin (g/dl)	3.5 (3.2–3.8)
Ferritin (ng/ml)	183 (85–355)
Transferrin saturation (%)	19 (14–25)
Phosphorus (mg/dl)	4.4 (3.7–5.5)
Parathyroid hormone (pg/ml)	225 (117–351)
IL-6 (pg/ml)	16.7 (7.3–39.2)
Myeloperoxidase (ng/ml)	37.5 (20.0–69.3)
Weekly EPO dose (units)	15,228 (7642–27,533)
Weekly EPO dose (units)/EDW	187 (98–385)

Categorical data are n (%). Continuous measures are mean ± SD. Laboratory values and erythropoietin (EPO) dose are median (quartile 1 to quartile 3). EDW, estimated dry weight of the patient in kilograms.

^aDiabetes mellitus includes all subjects with diabetic kidney disease as cause of ESRD.

data were assessed with chi-squared tests. Differences by mortality status for continuous clinical measures (mean and SDs) and laboratory characteristics (median and quartiles 1 and 3) were tested with independent samples *t* tests and Wilcoxon rank sum tests, respectively. Because of their positive skewness, ERI data were natural log-transformed, whereas %C-Alb was analyzed by quartile to facilitate interpretation of results (the lowest quartile used as the reference group). Parathyroid hormone level (pg/ml), ferritin (ng/ml), and IL-6 (pg/ml) were similarly log-transformed.

Univariate and multivariable linear regressions were used to evaluate the association between carbamylation quartile and natural log-ERI as the outcome. Laboratory data included in the multivariable analysis were measured concurrent to the ERI measure (*i.e.*, days 90–180 averages). Adjustments were made for 1-year mortality status (case-control status), and we tested interaction terms between carbamylation quartile and mortality status in all models. The interaction between carbamylation and mortality was not statistically significant in any model with ERI as the outcome (model with only carbamylation and mortality as covariates, $P=0.24$; multivariable model 1, $P=0.65$). Therefore, we did not stratify models by mortality status and analyzed the entire group of 158 individuals together as a cohort.

Multivariable model 1 included variables significant in univariate analysis at $P<0.10$ (specified *a priori*): baseline hemoglobin (g/dl), parathyroid hormone (pg/ml), albumin (g/dl), ferritin (ng/ml), transferrin saturation (%), having received intravenous iron therapy during the follow-up period (yes versus no), body mass index, and IL-6 (pg/ml). Model 2 added additional variables previously reported to influence EPO responsiveness: age, sex, race (white versus nonwhite), average urea reduction ratio, initial vascular

access (catheter versus no catheter), and an additional inflammatory marker myeloperoxidase (ng/ml). To assess linear trend across the quartile of carbamylation, the numeric values of the categorical variables were treated as scores and entered as a single continuous variable in the model (P value for trend).

In the secondary analysis, we evaluated the association between natural log-ERI (predictor) and 1 year all-cause mortality status (outcome) using univariate and multivariable logistic regression models. The latter included variables significant in univariate analysis at the $P<0.10$ level: vascular access, systolic BP (mmHg), diastolic BP (mmHg), albumin (g/dl), phosphorous (mg/dl), and IL-6 (pg/ml).

A sensitivity analysis was performed including all excluded participants ($n=29$) in the same models used for the primary and secondary analyses. We also performed a sensitivity analysis using ERI calculated from single time point hemoglobin and EPO dose measures taken on dialysis vintage day 90 (concurrent with the carbamylation measure as opposed to day 90–180 averages).

All statistics were performed using SAS (v9.2; SAS Institute, Cary, NC). Two-sided P values <0.05 were considered statistically significant, except for during covariate selection as described above.

Results

Baseline Characteristics

The baseline characteristics of the study population are summarized in Table 1. Study participants were predominantly Caucasian (67.1%), and the majority had a catheter as the initial vascular access (61.6%). Mean urea reduction ratio over the first 90 days of dialysis was 70.2% (SD=7.8). Relevant laboratory values included a median transferrin

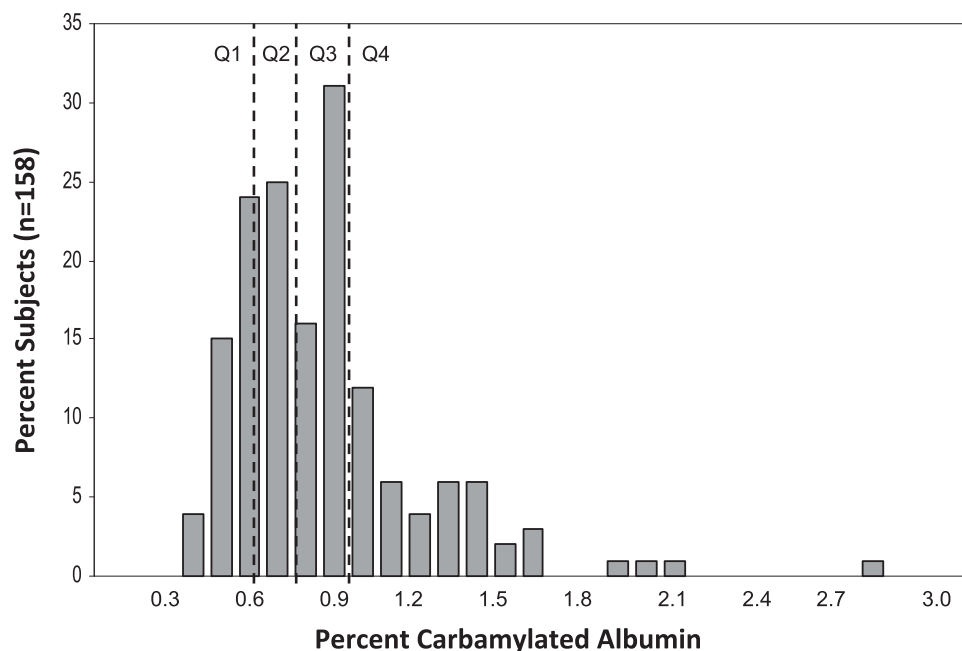


Figure 1. | Histogram of percent carbamylated albumin with quartiles. Distribution of percent carbamylated albumin levels in the study population ($n=158$). Dashed lines separate quartiles. Q1, lowest quartile; Q2, quartile 2; Q3, quartile 3; Q4, highest quartile.

Table 2. Associations to natural log-erythropoietin resistance index

Predictor	Univariate Analysis Difference in Log-ERI (95% CI)	P Value	Multivariable Model 1 Difference in Log-ERI (95% CI)	P Value	Multivariable Model 2 Difference in Log-ERI (95% CI)	P Value
Carbamylation						
Quartile 4 versus 1	0.51 (0.04 to 0.96)	0.03 ^a	0.55 (0.15 to 0.95)	0.01 ^b	0.66 (0.22 to 1.10)	0.004 ^c
Quartile 3 versus 1	0.08 (−0.37 to 0.54)	0.71 ^a	0.06 (−0.35 to 0.48)	0.75 ^b	0.20 (−0.27 to 0.67)	0.40 ^c
Quartile 2 versus 1	−0.29 (−0.74 to 0.16)	0.20 ^a	−0.21 (−0.60 to 0.19)	0.30 ^b	−0.16 (−0.59 to 0.28)	0.47 ^c
Age, yr	0.01 (−0.01 to 0.02)	0.43			−0.01 (−0.02 to 0.01)	0.63
Women	−0.11 (−0.44 to 0.22)	0.51			−0.24 (−0.58 to 0.10)	0.17
White race	−0.08 (−0.44 to 0.27)	0.64			0.06 (−0.26 to 0.39)	0.70
Urea reduction ratio	0.01 (−0.02 to 0.03)	0.65			0.01 (−0.03 to 0.03)	0.91
Hemoglobin (g/dl)	−0.32 (−0.43 to −0.22)	<0.001	−0.27 (−0.38 to −0.17)	<0.001	−0.27 (−0.38 to −0.16)	<0.001
Parathyroid hormone ^d (pg/ml)	−0.18 (−0.38 to 0.01)	0.07	−0.06 (−0.23 to 0.11)	0.48	−0.05 (−0.25 to 0.14)	0.58
Albumin (g/dl)	−1.0 (−1.42 to −0.65)	<0.001	−0.58 (−0.95 to −0.22)	0.002	−0.51 (−0.91 to −0.11)	0.01
Ferritin ^d (ng/ml)	−0.23 (−0.40 to −0.06)	0.01	−0.10 (−0.26 to 0.06)	0.23	−0.08 (−0.25 to 0.10)	0.38
Transferrin saturation (%)	−0.04 (−0.05 to −0.02)	<0.001	−0.03 (−0.04 to −0.01)	<0.001	−0.03 (−0.05 to −0.01)	<0.001
Received intravenous iron therapy	−0.43 (−0.87 to 0.01)	0.06	−0.27 (−0.64 to 0.10)	0.15	−0.26 (−0.68 to 0.16)	0.22
Body mass index	−0.04 (−0.06 to −0.02)	<0.001	−0.02 (−0.04 to −0.01)	0.01	−0.02 (−0.05 to 0.00)	0.04
Vascular access (catheter)	−0.26 (−0.61 to 0.08)	0.13			−0.08 (−0.42 to 0.27)	0.67
IL-6 ^d (pg/ml)	0.12 (−0.01 to 0.25)	0.06	−0.07 (−0.18 to 0.03)	0.18	−0.08 (−0.21 to 0.05)	0.22
Myeloperoxidase (ng/ml)	0.01 (−0.01 to 0.01)	0.62			0.01 (−0.00 to 0.01)	0.68

Effect estimates of the association between various predictors and the natural log-erythropoietin resistance index (log-ERI) in univariate and multivariable analysis. Difference in log-ERI (95% confidence interval [95% CI]) represents the difference in the log-ERI per unit change in the predictor (95% CI). Adjusted model 1 includes only variables significant in univariate analysis at $P<0.10$. Adjusted model 2 includes all variables from the univariate analysis.

^a P for overall trend across all quartiles=0.01.

^b P for overall trend across all quartiles=0.002.

^c P for overall trend across all quartiles=0.001.

^dVariable log-transformed.

saturation of 19% (interquartile range [IQR]=14–25) and a median ferritin of 183 ng/ml (IQR=85–355). At baseline, the median hemoglobin was 10.5 g/dl (IQR=9.6–11.3), and the median weekly EPO dose was 15,288 units (IQR=7642–27,533).

Carbamylation and EPO Resistance

The median %C-Alb was 0.77% (IQR=0.58–0.93) (Figure 1). Median ERI was 18.7 units/kg per grams per deciliter (IQR=8.1–35.6); 129 of 158 patients (81.6%) received intravenous iron during the follow-up period. We first examined the relationship between %C-Alb and ERI using linear regression and found that natural log-%C-Alb (linear) was positively associated with natural log-ERI (0.55 difference in log-ERI per unit change log-%C-Alb, $P=0.01$). Univariate analysis using quartiles of %C-Alb (categorical) showed ERI was 67% higher moving from the lowest quartile of %C-Alb to the highest quartile (0.51 increase in log-ERI from low to high quartile, $P=0.03$; P for trend=0.01) (Table 2).

In multivariable analysis including variables significant in the univariate models, the highest %C-Alb quartile showed a 72% higher ERI compared with the lowest quartile (0.55 increase in log-ERI from low to high quartile, $P=0.01$; P for trend=0.002) (Table 2, model 1). In addition to %C-Alb, baseline hemoglobin ($P<0.001$), albumin

($P=0.002$), transferrin saturation ($P<0.001$), and body mass index ($P=0.01$) were also associated with log-ERI. These results seemed similar after adding additional covariates to the model (Table 2, model 2).

Because albumin showed significance in the models and is included in the %C-Alb calculation, we tested for collinearity between albumin and %C-Alb in all models and found none (all variance inflation factors<2). Furthermore, %C-Alb and serum albumin concentration showed no significant correlation to each other ($R^2=-0.01$, $P=0.49$). Although our models adjusted for the urea reduction ratio, given the relationship of carbamylation to time-averaged urea, we also tested the correlation between %C-Alb and BUN level ($R^2=0.48$, $P<0.001$).

In the lowest carbamylation quartile, the median weekly EPO dose was 12,237 units (IQR=8554–25,565) over the course of 3 months. The median dose was significantly higher in the top carbamylation quartile at 18,044 units (IQR=10,325–43,318; top versus bottom quartile, $P=0.03$) (Figure 2). Despite a significantly higher EPO dose in the top carbamylation quartile, the median hemoglobin was lower (12.0 g/dl, IQR=10.8–13.0) compared with the bottom carbamylation quartile (12.7 g/dl, IQR=11.7–13.3; top versus bottom quartile, $P=0.01$). When analyzed in a continuous fashion, %C-Alb showed a positive correlation to

EPO dose ($R^2=0.20$, $P=0.01$) and a negative correlation to hemoglobin ($R^2=-0.25$, $P=0.001$) (Supplemental Figure 1).

In sensitivity analysis, using only single time point measures to analyze ERI (Supplemental Table 1) or including 29 excluded subjects into the analysis (Supplemental Figure 2 and Supplemental Tables 2 and 3) did not change the significance or direction of our findings.

EPO Resistance and Mortality

We examined if the ERI measure in our study could reproduce an association with mortality as others have described (2–4). Log-ERI was significantly associated with 1-year mortality in univariate analysis (odds ratio [OR] per unit increase in log-ERI value, 1.43; 95% confidence interval [95% CI], 1.02 to 2.02; $P=0.04$) (Figure 3). When the baseline characteristics of the study population were examined by 1-year mortality status, initial vascular access, baseline BP, serum albumin, phosphorous, and IL-6 level differed between groups at a $P<0.10$ level and thus, were included in multivariable analysis (Table 3). The ERI measure remained significantly associated with the risk of death in the multivariable model (OR per unit increase in the log-ERI value, 1.69; 95% CI, 1.06 to 2.70; $P=0.03$). However, the association between ERI and mortality was no longer statistically significant when %C-Alb was added to the multivariable analysis (ERI OR, 1.44; 95% CI, 0.87 to 2.37; $P=0.15$; compared with highest versus lowest quartile %C-Alb OR, 4.53; 95% CI, 1.20 to 17.1; $P=0.03$).

Discussion

In this study, the %C-Alb level in incident dialysis patients predicted EPO resistance, which was determined by the ERI. Although carbamylated albumin levels and ERI values both showed associations with mortality, adjusting for albumin carbamylation mitigated the risk of death associated with ERI. These observations suggest that protein carbamylation may be an important contributor to ERI as well as the association between EPO resistance and mortality. To our knowledge, this study is the first study to show these associations in humans. Although the direct pathogenic role of urea in ESRD is controversial (24), the relationship between protein carbamylation and EPO resistance is of interest, because it provides a mechanism linking uremia and EPO resistance. Carbamylation of EPO or proteins in the erythropoietic pathway may directly contribute to the pathophysiology of EPO resistance (12). Moreover, protein carbamylation may be a modifiable process, thus creating a potential therapeutic target (14,15,17).

Our study corroborates prior observations, in which carbamylation is associated with deleterious effects in various disease states (7,9,12,16,17,25–28). Although carbamylated EPO loses its erythropoietic properties both *in vitro* and *in vivo* (10,11), the timeframe for this process is unclear, and EPO dosing at every dialysis session provides uncarbamylated EPO routinely. Alternatively, several other proteins in the erythropoietic pathway, such as the EPO receptor, could become inhibited and drive EPO resistance. We chose to analyze carbamylated albumin as a surrogate of overall carbamylation burden, because it is an abundant and long-lived serum protein, even in dialysis patients

(15,29). Additional study will be required to ascertain whether carbamylation serves as a marker, effector, or both of EPO resistance.

Although we observed a correlation between BUN and carbamylation, carbamylated albumin may be a more accurate measure of urea load than BUN, which fluctuates 40%–70% between dialysis sessions (30). This relationship would be analogous to measuring glycated hemoglobin A1C to determine time-averaged glucose control in diabetes mellitus. Increasing dialysis adequacy or the urea reduction value can result in an increase in the response to EPO in ESRD patients (31,32), although we did not see such an association in our study. This result is perhaps because the mean urea reduction ratio at baseline in our study was high (70%) (31), and by 6 months, it rose even higher (72%), indicating that few, if any, participants were underdialyzed.

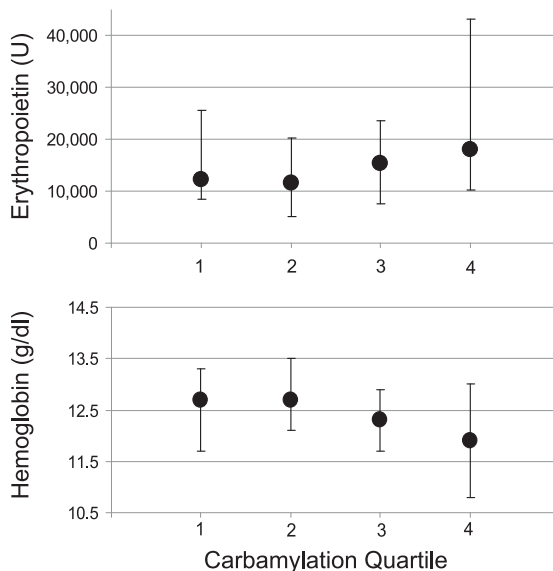


Figure 2. | Erythropoietin dose and hemoglobin by quartile of carbamylation. Median weekly erythropoietin dose (units) and median hemoglobin (g/dl) values between days 90 and 180 across quartile of percent carbamylated albumin. Bars are interquartile range.

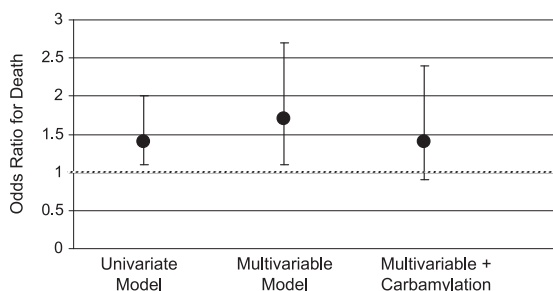


Figure 3. | Odds ratio for death by erythropoietin resistance index. Odds ratios represent odds per unit change in natural log-erythropoietin resistance index. Bars are 95% confidence interval. Multivariable model includes systolic BP, diastolic BP, vascular access (catheter versus no catheter), albumin, phosphorous, and IL-6.

Table 3. Baseline characteristics by 1-year mortality status in the study population (secondary analysis)

Variable	Subjects Alive at 1 yr (n=106)	Subjects Dead at 1 yr (n=52)	P Value ^a
Age, yr	69.5±12.7	70.3±13.1	0.70
Women	51 (48.1)	30 (57.7)	0.26
White race	73 (68.9)	33 (63.5)	0.50
Comorbidities			
Diabetes mellitus	31 (29.3)	12 (23.1)	0.41
Coronary artery disease	13 (12.3)	7 (13.5)	0.83
Congestive heart failure	20 (18.9)	13 (25.0)	0.37
Vascular access: catheter	58 (56.9)	35 (71.4)	0.08
Body mass index (kg/m ²)	26.5±7.2	27.7±7.0	0.29
Systolic BP (mmHg)	147.8±22.5	138.9±26.6	0.03
Diastolic BP (mmHg)	73.7±11.6	69.4±14.4	0.04
Urea reduction ratio	68.3±10.6	68.7±7.8	0.85
Laboratory data			
Hemoglobin (g/dl)	10.5 (9.7–11.3)	10.5 (9.6–11.2)	0.44
Albumin (g/dl)	3.5 (3.3–3.9)	3.4 (3.2–3.7)	0.05
Ferritin (ng/ml)	183 (100–324)	181 (79–402)	0.76
Transferrin saturation (%)	19 (15–25)	17 (13–24)	0.22
Phosphorus (mg/dl)	4.6 (3.8–5.7)	4.2 (3.2–5.1)	0.03
Parathyroid hormone (pg/ml)	221 (115–318)	230 (117–473)	0.18
IL-6 (pg/ml)	11.9 (6.0–31.0)	24.8 (12.0–46.5)	0.01
Myeloperoxidase (ng/ml)	35.4 (19.6–69.6)	40.4 (20.4–63.8)	0.60

Categorical data are *n* (%). Continuous measures are mean ± SD. Laboratory values are median (quartile 1 to quartile 3). Values reflect baseline measurements taken during the first 90 days of initiating dialysis.

^a*P* values represent alive versus dead chi-squared, Wilcoxon rank sum, or *t* tests as appropriate.

It is also possible that carbamylation reflects a high inflammatory state, which is known to lead to EPO resistance through multiple mechanisms (1,33,34). Our data included measurements of the inflammatory markers IL-6 and myeloperoxidase as well as albumin and ferritin, which have been shown to correlate with inflammation and EPO resistance (35,36). In univariate analyses, we observed associations between EPO resistance and albumin, ferritin, and IL-6 levels, but in multivariable analyses, carbamylated albumin remained strongly associated with EPO resistance. This result suggests that protein carbamylation either participates in the association between chronic inflammation and EPO resistance or has a dominant influence on ERI compared with chronic inflammation.

To quantify EPO resistance, ERI has been successfully used as a single variable incorporating two principle components considered in EPO dosing: the weight of the patient and the level of anemia despite treatment (1,35,37). Others have suggested that cumulative EPO dosing may also be suitable in such studies (38). Our results did not change, regardless of the outcome that we analyzed (Figure 2 and Supplemental Table 1 show alternatives).

We also showed that, although ERI was associated with mortality, carbamylation seemed to be a stronger predictor of death. Although carbamylation is a reliable predictor of ESRD mortality, the mechanisms are not clearly established, and our data cannot discern if carbamylation participates directly in the association between EPO resistance and mortality or simply has an overriding effect relative to EPO resistance (15,16). Interestingly, studies from tissue culture and animal models suggest that protein carbamylation may contribute to cardiovascular

disease. For example, carbamylated proteins, such as LDL, can induce endothelial cell death, vascular smooth muscle proliferation, monocyte inflammatory signaling, and endothelial/monocyte cell adhesion—each potentially accelerating the biochemical events of atherosclerosis (8,9,14,39,40). Moreover, carbamylated albumin itself seems to be proinflammatory (41). Additional study will need to clarify the mechanisms linking carbamylation with ESRD mortality.

Our study has certain limitations. The observational design precludes discussions of causality, and we acknowledge that our findings are hypothesis-generating. We had only a single time measure of carbamylation. However, the carbamylated albumin test provides an index of protein carbamylation over the lifespan of circulating albumin, thus offering an extended, time-averaged assessment (15). Because the initial dataset stemmed from a case-control mortality study, there was concern for confounding by mortality status. However, the interaction between carbamylation and mortality was not significant for any model predicting ERI. Furthermore, including mortality status as a covariate did not alter the statistical significance of the results associating carbamylation and ERI, suggesting that any residual confounding by 1-year mortality was minimal. Lastly, the sample size was relatively small. The sensitivity analysis performed—replicating the regression models but including 29 excluded subjects—showed that the relationship between %C-Alb and ERI was only strengthened (Supplemental Table 2). Likewise, the results of the mortality analysis did not vary significantly when 29 early deaths were included (Supplemental Figure 1 and Supplemental Table 3).

In conclusion, carbamylated albumin at 3 months from dialysis initiation is associated with subsequent ERI, even after accounting for traditional variables influential of EPO responsiveness. Although ERI was verified as a predictor of mortality, carbamylation was a stronger predictor of death. The link between carbamylation and EPO resistance may allow us to better understand the pathophysiology of anemia in ESRD and how best to treat patients affected by it.

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Disclosures

Provisional applications for US and International Patents related to the contents of this manuscript have been filed by A.H.B., S.A.K., and R.I.T. and their affiliated institutions. R.I.T. is a consultant to Fresenius Medical Care North America.

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