

Clinical Measures Identify Vitamin D Deficiency in Dialysis

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Background and objectives: Vitamin D deficiency (defined by serum levels of 25-hydroxyvitamin D) is common in patients with ESRD on hemodialysis, but risk factors are unknown. This study was conducted to determine whether routinely measured clinical and demographic parameters could identify dialysis patients who are vitamin D deficient.

Design, setting, participants, & measurements: Nine-hundred eight patients with 25-hydroxyvitamin D levels were identified from the Accelerated Mortality on Renal Replacement (ArMORR) cohort of incident U.S. dialysis patients and were divided into training (60%) and validation (40%) sets. Predictive models were generated from routinely assessed clinical and demographic data in the training set using logistic regression modeling, neural networks, and decision trees with vitamin D deficiency as the dependent variable. Models underwent progressive variable reduction to identify the simplest model that remained predictive.

Results: Seventy-nine percent of the population was vitamin D deficient (25-hydroxyvitamin D <30 ng/ml). Black race, female sex, winter season, and hypoalbuminemia (serum albumin \leq 3.1 g/dl) were the strongest predictors of vitamin D deficiency. In the validation set, the presence of hypoalbuminemia and winter season increased the likelihood of vitamin D deficiency in black women (from 90% to 100%), black men (from 85% to 100%), white women (from 82% to 94%), and white men (from 66% to 92%).

Conclusions: Deficiency of 25-hydroxyvitamin D is nearly universal among patients with hypoalbuminemia initiating chronic hemodialysis in winter.

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Impaired metabolism of vitamin D is among the most recognized disorders associated with chronic kidney disease (CKD). In healthy individuals, vitamin D is initially synthesized in the skin or acquired via the diet, hydroxylated to 25-hydroxyvitamin D in the liver, then converted to 1,25-dihydroxyvitamin D, principally in the kidney (1). In patients with CKD, the production of 1,25-dihydroxyvitamin D decreases in concert with declining renal function, a phenomenon primarily attributed to a decline in the activity of the renal 1α -hydroxylase enzyme (2). 1,25-dihydroxyvitamin D is considered to be the active form of vitamin D and thus has been the focus of management guidelines for ESRD, (3) whereas 25-hydroxyvitamin D is the form that reflects dietary intake or sun exposure. Serum levels of 25-hydroxyvitamin D are used to define vitamin D deficiency in the general population (4).

However, recently it has been shown that 25-hydroxyvitamin D can be converted to 1,25-dihydroxyvitamin D at sites other than the kidney, including the prostate, breast, colon, and macrophages (5,6). Local production of 1,25-dihydroxyvitamin D may be important for several biologic functions in these tissues; thus, circulating 25-hydroxyvitamin D levels may be relevant

even when renal production of 1,25-dihydroxyvitamin D is low. Levels of cathelicidin, an antimicrobial peptide regulated by local vitamin D conversion in macrophages, correlate with susceptibility to death due to infectious causes in hemodialysis patients (7). Furthermore, low levels of 25-hydroxyvitamin D are associated with increased mortality in ESRD (8,9). Thus, there are compelling epidemiologic and basic science data that suggest that guidelines for identifying and treating 25-hydroxyvitamin D deficiency in patients with ESRD should be revised.

25-hydroxyvitamin D levels are not routinely measured in ESRD, but evidence suggests that 50% to 90% of this population is deficient; despite its high cost, testing is becoming increasingly prevalent (8,10 to 12). Studies of the general population have identified age, race, season, smoking, and body mass index as risk factors for vitamin D deficiency (13–17). However, similar analyses have not been conducted with dialysis patients, who are at additional risk of deficiency because of factors such as impaired photoproduction of vitamin D and altered levels of vitamin D binding protein and megalin (18,19). We used an existing cohort of incident dialysis patients to test the hypothesis that commonly collected clinical characteristics could predict the risk of vitamin D deficiency.

Materials and Methods

Accelerated Mortality on Renal Replacement (ArMORR) is a nationally representative prospective cohort study of incident chronic hemodialysis patients who began renal replacement between July 1, 2004 and

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Table 1. Baseline characteristics of the ArMORR cohort

Age, years	64 (52 to 75)
Gender, % male	53.0
Race, %	
white	61.0
black	32.0
other	7.0
Cause of ESRD, %	
diabetes mellitus	43.8
hypertension	33.7
glomerulonephritis	9.5
polycystic kidney disease	2.6
other	10.4
Initiated dialysis as inpatient, %	22.5
Systolic blood pressure, mmHg	144 (130 to 159)
Diastolic blood pressure, mmHg	74 (65 to 83)
Body mass index	26 (22 to 31)
Albumin, g/dl	3.5 (3.1 to 3.8)
Calcium, mg/dl	8.5 (8.0 to 9.0)
Phosphorus, mg/dl	4.5 (3.6 to 5.6)
Hemoglobin, g/dl	10.3 (9.4 to 11.2)
Ferritin, ng/ml	202 (98 to 398)
White blood count, th/cmm ^a	8.0 (6.3 to 10.1)
PTH, pg/ml	206 (111 to 352)
25-hydroxyvitamin D, ng/ml	18.2 (11.3 to 28.0)

Demographic, clinical, and laboratory data were determined within the first 14 days of initiating dialysis. Continuous measures are reported as median and interquartile range.

^ath/cmm, thousands per cubic millimeter.

July 30, 2005 at 1 of 1056 dialysis centers in the United States operated by Fresenius Medical Care, North America (7). The ArMORR data set contains a broad range of demographic and clinical data including medical problems, laboratory results, as well as serum and plasma samples. Samples were obtained at the initiation of dialysis. Clinical data were collected prospectively and entered uniformly into a central database by practitioners at the point of care. All clinical data arriving at Fresenius undergo rigorous data quality assurance and quality control auditing. Blood samples collected for clinical care were shipped to and processed by Spectra East (Rockland, NJ). After processing for routine clinical testing, remnant samples were shipped on ice to the ArMORR investigators where the samples were aliquotted and stored in liquid nitrogen tanks. This study was approved by the Institutional Review Board of the Massachusetts General Hospital and conducted in accordance with its ethical standards.

Study Population

Between July 1, 2004 and June 30, 2005, 10,044 incident hemodialysis patients were prospectively enrolled into ArMORR. A random sample of 908 patients had 25-hydroxyvitamin D levels that had previously been measured for research purposes and had demographic characteristics that were representative of the larger cohort (Table 1) (8). The sample was randomly divided into training and validation sets for the predictive models. Randomization was performed in SAS 9.1.3 (SAS Institute, Cary, NC).

Assays

25-hydroxyvitamin D levels were measured at a single laboratory from baseline (within 14 days of dialysis initiation) samples using commercially available RIA techniques (DiaSorin Inc., Stillwater, MN). The coefficients of variation for 25-hydroxyvitamin D measurements were <3% at levels <30 ng/ml and 9.4% at levels <10 ng/ml. Albumin was measured on an Olympus 5400 using a dye binding assay. Calcium and phosphorous were measured on an Olympus 5400 using a colorimetric assay. Ferritin and parathyroid hormone (PTH) were measured on an Advia Centaur using a chemiluminescence enzyme immunoassay. Hemoglobin and white blood counts were measured were measured on an Advia 120 using colorimetric and flow cytometric assays, respectively.

Building a Prediction Model

The study population was randomly partitioned into training and validation sets, comprising 60% and 40% of subjects, respectively. SAS Enterprise Miner 4.3 (SAS Institute, Cary, NC) was used to algorithmically build a predictive model from the subjects in the training set. Various thresholds have been used to define vitamin D deficiency in the literature and to link deficiency to clinical outcomes. To cover the

Table 2. Clinical and demographic variables

Demographic
age
race
gender
Clinical
body mass index
diabetes
coronary artery disease
peripheral vascular disease
stroke
congestive heart failure
hypertension
chronic obstructive pulmonary disease
cancer
liver disease
systolic and diastolic blood pressure
hemodialysis access
Laboratory data
albumin
bicarbonate
calcium
ferritin
hemoglobin
phosphorus
PTH
white blood count
Situational
initiation site (inpatient/outpatient)
season
latitude

These variables were included as predictor variables for the model-building algorithms.

scope of these definitions, we included three commonly used thresholds: 30 ng/ml (4,8,9,20,21) 20 ng/ml (4,14,22,23), and 10 ng/ml (8,24–26).

We included basic demographic characteristics that might influence vitamin D levels, available clinical characteristics including documented comorbidities that might affect access to sunlight (obtained from form 2728), standard baseline laboratories typically obtained at dialysis initiation, and situational information [including season and latitude, which can affect ultraviolet (UV) light exposure and thus vitamin D production]. Candidate covariates are listed in Table 2. Laboratory data were obtained within 14 days of initiating dialysis. Season was coded as winter (October to March) or summer (April to September). A four-category variable was used to identify the latitude of the state where each patient received dialysis. Continuous variables were converted to three binary variables using the 25th percentile, the median, and the 75th percentile as cutoffs. Variables were removed if they were not significantly correlated with target variable, on the basis of an $R^2 < 0.5$. The remaining variables were then processed by applying three popular methods for generating predictive models available through SAS Enterprise Miner (SAS Institute, Cary, NC): multivariate logistic regression, neural network modeling, and decision tree learning.

Multivariate regression allows simultaneous assessment of the relationship between multiple potential predictors and a binary outcome variable but does not address interactions between predictors unless these are specifically examined (27). Artificial neural networks are nonlinear models thought to be patterned after the human brain. They are capable of detecting complex relationships between predictors and the outcome variable, including multiple interactions between predictors, but can be more difficult to interpret and apply in clinical practice

than regression models (28,29). Decision trees (also known as classification and regression trees) are also sensitive to interaction between predictors; models are presented in a flowchart-like structure, with the population divided into smaller and smaller subgroups, which makes them easily adaptable to clinical practice (27,30–32). We assessed all three methods to identify the best model for this application.

For regression and neural network analysis, missing laboratory values were replaced with the most frequent value among the study population; missing values were present in $<5\%$ of subjects for all variables except for PTH (10%) and ferritin (6%). All modeling techniques used the standard settings supplied by SAS Enterprise Miner: regression models used stepwise selection; neural networks used misclassification rate, one three-unit hidden layer of three units, and a weight decay of 0; and tree modeling used χ^2 test with a significance cutoff of $P = 0.2$ as a splitting criterion with a minimum of ten observations per leaf.

Model Assessment

After predictive modeling, the performance of each algorithm was assessed by receiver operating characteristic curves. On the basis of the area under the curve (or C-statistic), we selected the best algorithm for assessment in the validation set.

To facilitate interpretability of regression models, we tested these models in the validation set using a prediction score, with one point given for the presence of each predictor. After evaluating the score variable's performance in the testing set, the least predictive variable was serially removed from the score. After each variable was removed, the C-statistic was reassessed to measure the effect on the predictive value. The model that remained most stable after variable reduction was selected as the final model.

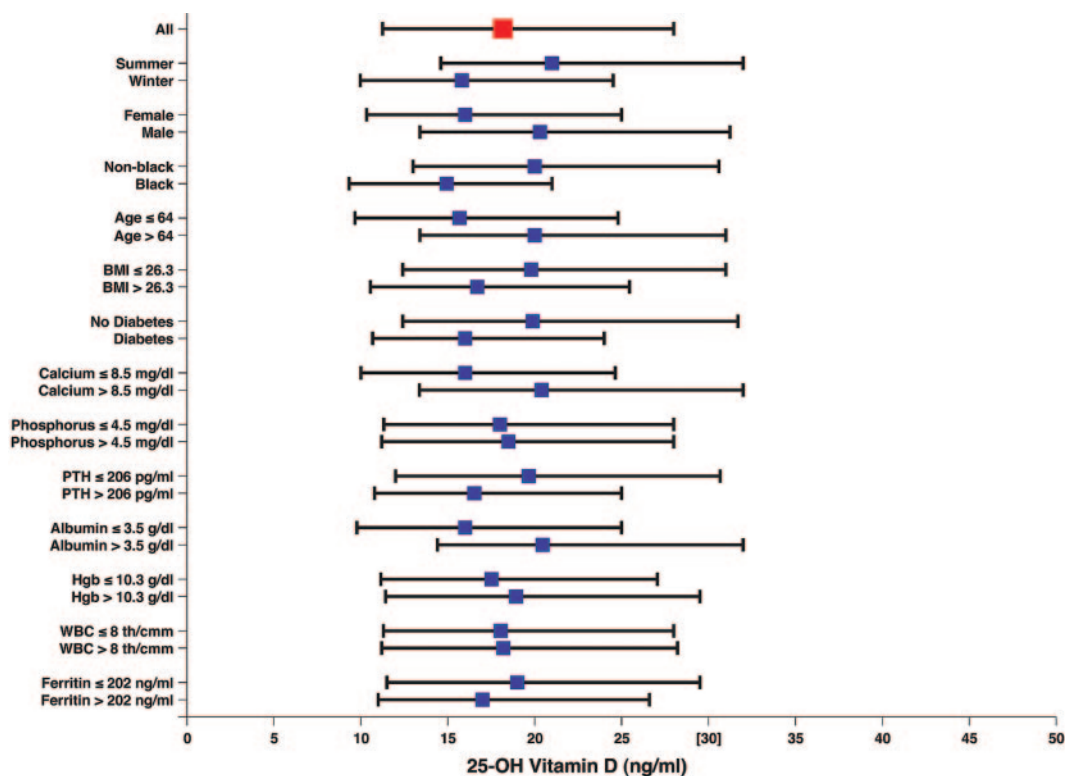


Figure 1. Distribution of 25-hydroxyvitamin D levels. The median 25-hydroxyvitamin D for the entire population (red) and for individuals with specific characteristics (blue) are presented along with the interquartile range for each group. Subgroups for continuous variables are defined based on population medians.

Results

A total of 908 subjects residing in 37 different states were included in the analysis. Using the different thresholds for vitamin D deficiency, 79% of subjects had 25-hydroxyvitamin D levels <30 ng/ml, whereas 57% and 20% had levels <20 and 10 ng/ml, respectively. The distribution of 25-hydroxyvitamin D levels on the basis of patient characteristics is presented in Figure 1. At each of the three 25-hydroxyvitamin D thresholds used for defining the outcome variable (vitamin D deficiency), regression and neural network models were superior to decision trees, with higher training-set C-statistics (Table 3). Given the superior interpretability and performance of regression models, these models were used for subsequent analysis.

Predictors Identified

The regression model predictors (identified by stepwise selection) are displayed in Figure 2; seven predictors were identified when the model was developed using a threshold for vitamin D deficiency of 30 ng/ml (model 1) or 20 ng/ml (model 2), and five predictors were selected for a threshold of 10 ng/ml (model 3). For all models, initiating chronic hemodialysis in the winter, having a low serum albumin, and being of black race each increased the likelihood of vitamin D deficiency. Low albumin levels and winter initiation were among the top four most powerful predictors of vitamin D deficiency in all three models. Female sex and diabetes were predictors in models 1 and 2, whereas a serum calcium level below the median (uncorrected level ≤ 8.5 mg/dl) was a significant predictor in models 2 and 3.

Model Performance

When tested in the validation set, models 1 and 2 (which had C-statistics of 0.70) were superior to model 3 (C-statistic of 0.65). To identify the most parsimonious model that remained predictive, all models were reassessed after serial removal of the least predictive variables (Figure 3). Model 2 proved the most resilient, with a decrease in C-statistic from 0.70 to 0.69 after the removal of the three variables. This simplified version of model 2 identified black race, female sex, winter season, and hypoalbuminemia (serum albumin in the lowest quartile, ≤ 3.1 g/dl) as being predictive of vitamin D deficiency. One-hundred

Table 3. Relative performance of different approaches to generating predictive models for vitamin D deficiency

Threshold (ng/ml)	Regression	Neural Network	Decision Tree
30	0.8	0.79	0.73
20	0.79	0.78	0.73
10	0.8	0.79	0.73

Values represent the training-set C-statistic for each modeling technique when using different thresholds of 25-hydroxyvitamin D to define deficiency. C-statistic is a measure of the model's ability to correctly predict the outcome (a model with a C-statistic of 1 would always predict the correct outcome, whereas a C-statistic of 0.5 would be of no predictive value) (51).

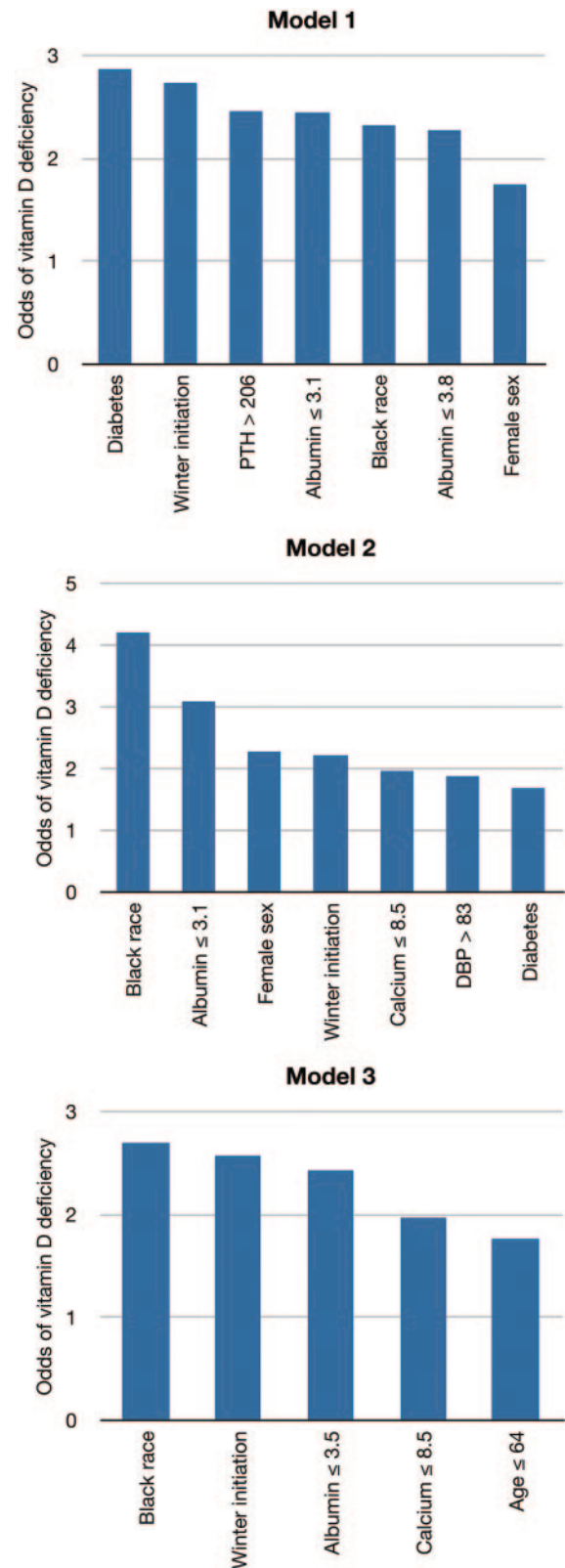


Figure 2. Regression models for vitamin D deficiency generated from the training set using thresholds defining deficiency as 25-hydroxyvitamin D <30 ng/ml, <20 ng/ml, and <10 ng/ml for models 1, 2, and 3, respectively. Black race, albumin, and season were identified as predictors in each of the three models.

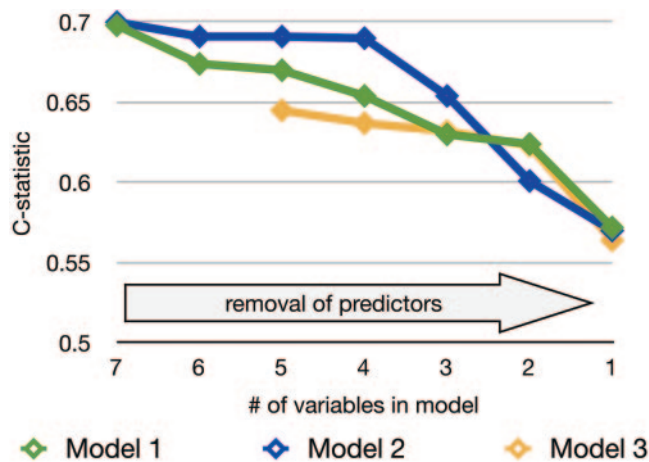


Figure 3. Decline in predictive power of regression models to predict vitamin D deficiency after stepwise removal of the least predictive variables. At each stage, the weakest remaining predictor was removed from the model and its predictive power was reassessed. Model 2 proved most resilient to variable reduction and its top four predictors (race, sex, season, and albumin) were used in the final model.

percent of black women with an albumin ≤ 3.1 g/dl who initiated hemodialysis during the winter had 25-hydroxyvitamin D levels < 20 ng/ml. Of the 10% of the population that had none of these characteristics, 78% of patients had 25-hydroxyvitamin D levels ≥ 20 ng/ml.

Race and sex were the most powerful demographic predictors of 25-hydroxyvitamin D deficiency; thus, we assessed the ability of the remaining predictors (winter season and hypoalbuminemia) to identify vitamin D deficiency within each category of race and sex (Figure 4). Regardless of the 25-hydroxyvitamin D threshold used to define deficiency, winter season and hypoalbuminemia increased the likelihood of iden-

tifying vitamin D deficiency. All black patients with hypoalbuminemia initiating dialysis in the winter had 25-hydroxyvitamin D levels < 20 ng/ml. In white female patients, 94% of those with these two characteristics had levels < 30 ng/ml and 88% had levels < 20 ng/ml (compared with baseline prevalence of 82% and 62%, respectively). White male patients had lower baseline rates of vitamin D deficiency using thresholds of either 30 ng/ml (66%) or 20 ng/ml (44%), but these were also increased in setting of hypoalbuminemia and winter season (92% and 67%, respectively). In the overall population, these two predictors alone were highly specific for vitamin D deficiency (Table 4).

Discussion

Using a cohort of incident dialysis patients, we identified clinical and demographic characteristics that increased the likelihood of vitamin D deficiency. In the entire cohort, the prevalence of the deficiency was high, and in subjects with hypoalbuminemia initiating hemodialysis in winter, nearly all subjects (95%) had levels < 30 ng/ml and 83% had levels < 20 ng/ml. This relationship was particularly strong in black patients, in whom levels < 20 ng/ml were universal when hypoalbuminemia was present and dialysis was initiated in the winter. The presence of hypoalbuminemia, particularly in women and black men, may reduce the need to measure serum levels of 25-hydroxyvitamin D in patients initiating dialysis between October and March. Given the high cost of 25-hydroxyvitamin D testing relative to treatment, empiric therapy with a nutritional form of vitamin D (*e.g.*, ergocalciferol 50,000 IU monthly) could be considered in these individuals if our results are validated by other investigators (11).

Black race was the leading independent predictor of vitamin D deficiency when definitions were based on a 25-hydroxyvitamin D level < 10 or < 20 ng/ml, and remained a significant predictor using a definition of < 30 ng/ml; this association has

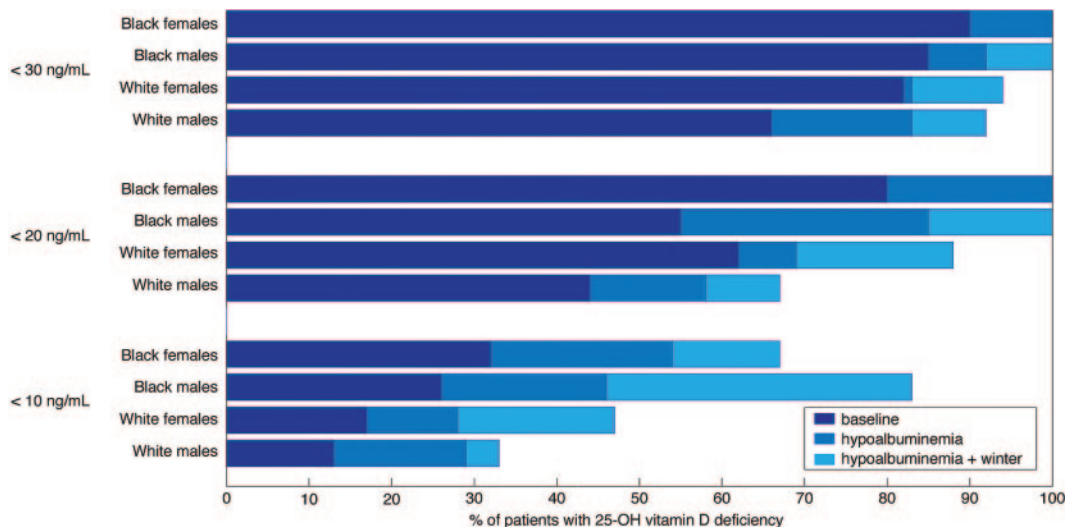


Figure 4. Likelihood of vitamin D deficiency at baseline, with hypoalbuminemia, and with hypoalbuminemia and winter season. Regardless of 25-hydroxyvitamin D level used to define deficiency (< 10 ng/ml, < 20 mg/ml, or < 30 ng/ml), individuals who initiated dialysis in winter and were hypoalbuminemic (serum albumin ≤ 3.1 g/dl) were more likely to be vitamin D deficient.

Table 4. Predicting vitamin D deficiency with albumin and season

25-Hydroxyvitamin D Level	Predicting Deficiency (albumin \leq 3.1 mg/dl, winter)			
	Sensitivity (%)	Specificity (%)	PPV ^a (%)	NPV ^b (%)
<30 ng/ml	14	97	95	23
<20 ng/ml	17	95	83	45
<10 ng/ml	29	93	50	84

Subjects with serum albumin in the lowest quartile (\leq 3.1 g/dl) who initiated dialysis in winter were more likely to be vitamin D deficient, regardless of the threshold of 25-hydroxyvitamin D used. This relationship had a high specificity, particularly for a threshold of 30 ng/ml.

^aPPV, positive predictive value.

^bNPV, negative predictive value.

also been observed in the general population (16,17,25). Melanin absorbs UV light and reduces the energy available for conversion of 7-dehydrocholesterol to previtamin D (1). Initiation of dialysis during winter was also a powerful predictor of vitamin D deficiency regardless of cutoff, likely because of decreased UV exposure during the winter months (16,17,33). Previous studies have found that photoproduction of vitamin D is markedly impaired in ESRD, suggesting that dietary sources of vitamin D would be the major determinant of 25-hydroxyvitamin D levels in this population (18).

Low serum albumin levels were also associated with an increased risk of vitamin D deficiency in this analysis. Albumin is likely to reflect level of nutrition, and low serum albumin might correlate with lower intake of dietary sources of vitamin D. Future studies could clarify this relationship by examining if other measures of nutrition (*e.g.*, normalized protein nitrogen appearance) also predict with vitamin D deficiency. Alternatively, low albumin could represent reduced carrying capacity for vitamin D, which largely circulates in protein-bound form (34). However, another possible explanation is that a common disorder predisposes to hypoalbuminemia and vitamin D deficiency. This could represent chronic inflammation, an aspect of many comorbid illnesses, which may also be associated with decreased UV exposure; however, further adjustment for several documented comorbidities (coronary artery disease, hypertension, chronic obstructive pulmonary disease, cancer, peripheral vascular disease, stroke, congestive heart failure, and liver disease), white blood count, and ferritin did not materially alter the relationship of albumin with vitamin D deficiency. Another such mechanism might be nephrotic syndrome, which is associated with urinary losses of albumin and may also contribute to loss of vitamin D binding protein, the major carrier protein for vitamin D (35,36).

Other associations we observed might be effects caused by, rather than causes of, vitamin D deficiency. Because vitamin D is needed for calcium absorption, the observation that vitamin D deficiency was associated with low calcium levels and high PTH is consistent with known physiology in individuals without kidney disease; however, the observed relationship with calcium was no longer significant when these levels were corrected for serum albumin. Given the impairment in 1α -hydroxylase activity that occurs with CKD, 25-

hydroxyvitamin D levels have previously been thought to be a poor reflection of the traditional effects of vitamin D on mineral metabolism. Prior studies have identified a correlation between 25-hydroxyvitamin D and PTH principally in early CKD (37,38). Our finding of an association with PTH, echoing an earlier study (39), suggests that levels of 25-hydroxyvitamin D are also important in ESRD, even with respect to traditional actions on calcium homeostasis.

The significance of 25-hydroxyvitamin D levels in ESRD is not yet completely understood, but deficiency has been observed in most individuals on dialysis (2,8,26,40). Studies associating active vitamin D treatment with improved survival in dialysis and predialysis CKD are now numerous (41–47). In hemodialysis patients, 25-hydroxyvitamin D levels have been shown to correlate with all-cause and cardiovascular mortality (8). Although renal 1α -hydroxylase activity declines as CKD advances, the same may not be true of the extrarenal enzyme (40,48). This activity may be particularly important in the immune system, where production of the endogenous antimicrobial peptide cathelicidin is regulated by local conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D (6). Indeed, low circulating levels of cathelicidin have recently been shown to correlate with increased mortality due to infectious causes in dialysis (7).

Although our cohort represents a broad range of dialysis units throughout the United States, (49) it is not clear if these findings are generalizable to populations outside of the United States. The direction of causality in the observed associations is uncertain, and residual confounding cannot be excluded. Additional investigation is needed to determine if repletion of 25-hydroxyvitamin D affects hyperglycemia, blood pressure, infection rates, or mortality rates in ESRD. Although this study identified clinical factors that predicted low 25-hydroxyvitamin D levels, it is not yet proven that correcting these levels is clinically beneficial. Prospective studies in ESRD, some of which are now underway, (50) are needed to identify optimal levels of 25-hydroxyvitamin D for a range of functions and to further elucidate its biology. In the absence of clinical trials, clinicians must independently determine if these findings should guide empiric therapy or simply inform future studies.

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References

- Chen TC, Chimeh F, Lu Z, Mathieu J, Person KS, Zhang A, Kohn N, Martinello S, Berkowitz R, Holick MF: Factors that influence the cutaneous synthesis and dietary sources of vitamin D. *Arch Biochem Biophys* 460: 213–217, 2007
- Ishimura E, Nishizawa Y, Inaba M, Matsumoto N, Emoto M, Kawagishi T, Shoji S, Okuno S, Kim M, Miki T, Morii H: Serum levels of 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitamin D, and 25-hydroxyvitamin D in nondialyzed patients with chronic renal failure. *Kidney Int* 55: 1019–1027, 1999
- Foundation NK: K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 42: S1–S201, 2003
- Holick MF: Vitamin D deficiency. *N Engl J Med* 357: 266–281, 2007
- Reichel H, Koeffler HP, Norman AW: Synthesis in vitro of 1,25-dihydroxyvitamin D₃ and 24,25-dihydroxyvitamin D₃ by interferon-gamma-stimulated normal human bone marrow and alveolar macrophages. *J Biol Chem* 262: 10931–10937, 1987
- Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schaubert J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL: Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311: 1770–1773, 2006
- Gombart AF, Bhan I, Borregaard N, Tamez H, Camargo CA Jr, Koeffler HP, Thadhani R: Low plasma level of cathelicidin antimicrobial peptide (hCAP18) predicts increased infectious disease mortality in patients undergoing hemodialysis. *Clin Infect Dis* 48: 418–424, 2009
- Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, Steele D, Chang Y, Camargo CA Jr, Tonelli M, Thadhani R: Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int* 72: 1004–1013, 2007
- Ravani P, Malberti F, Tripepi G, Pecchini P, Cutrupi S, Pizzini P, Mallamaci F, Zoccali C: Vitamin D levels and patient outcome in chronic kidney disease. *Kidney Int* 75: 88–95, 2009
- London GM, Guérin AP, Verbeke FH, Pannier B, Boutouyrie P, Marchais SJ, Métivier F: Mineral metabolism and arterial functions in end-stage renal disease: Potential role of 25-hydroxyvitamin D deficiency. *J Am Soc Nephrol* 18: 613–620, 2007
- Saab G, Young DO, Gincherman Y, Giles K, Norwood K, Coyne DW: Prevalence of vitamin D deficiency and the safety and effectiveness of monthly ergocalciferol in hemodialysis patients. *Nephron Clin Pract* 105: c132–138, 2007
- Blair D, Byhamgray L, Lewis E, Mccaffrey S: Prevalence of vitamin D [25(OH)D] deficiency and effects of supplementation with ergocalciferol (vitamin D₂) in stage 5 chronic kidney disease patients. *J Ren Nutr* 18: 375–382, 2008
- Holick MF: High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* 81: 353–373, 2006
- Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ: Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med* 158: 531–537, 2004
- Benjamin A, Moriakova A, Akhter N, Rao D, Xie H, Kukreja S, Barendolts E: Determinants of 25-hydroxyvitamin D levels in African-American and Caucasian male veterans. *Osteoporos Int* 20: 1795–1803, 2009
- Bodnar LM, Catov JM, Wisner KL, Klebanoff MA: Racial and seasonal differences in 25-hydroxyvitamin D detected in maternal sera frozen for over 40 years. *Br J Nutr* 101: 278–284, 2009
- Hirani V, Mosdøl A, Mishra G: Predictors of 25-hydroxyvitamin D status among adults in two British national surveys. *Br J Nutr* 101: 760–764, 2009
- Jacob AI, Sallman A, Santiz Z, Hollis BW: Defective photoproduction of cholecalciferol in normal and uremic humans. *J Nutr* 114: 1313–1319, 1984
- Dusso AS, Brown AJ, Slatopolsky E: Vitamin D. *Am J Physiol Renal Physiol* 289: F8–F28, 2005
- Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R: Estimates of optimal vitamin D status. *Osteoporos Int* 16: 713–716, 2005
- Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B: Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 84: 18–28, 2006
- Malabanan A, Veronikis IE, Holick MF: Redefining vitamin D insufficiency. *Lancet* 351: 805–806, 1998
- Holick MF, Chen TC, Lu Z, Sauter E: Vitamin D and skin physiology: a D-lightful story. *J Bone Miner Res* 22[Suppl 2]: V28–V33, 2007
- Holick MF: The use and interpretation of assays for vitamin D and its metabolites. *J Nutr* 120[Suppl 11]: 1464–1469, 1990
- Ginde AA, Liu MC, Camargo CA: Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. *Arch Intern Med* 169: 626–632, 2009
- LaClair RE, Hellman RN, Karp SL, Kraus M, Ofner S, Li Q, Graves KL, Moe SM: Prevalence of calcidiol deficiency in CKD: A cross-sectional study across latitudes in the United States. *Am J Kidney Dis* 45: 1026–1033, 2005
- Grobman WA, Stamilio DM: Methods of clinical prediction. *Am J Obstet Gynecol* 194: 888–894, 2006
- Tu JV: Advantages and disadvantages of using artificial neural networks versus logistic regression for predicting medical outcomes. *J Clin Epidemiol* 49: 1225–1231, 1996
- Tafeit E, Reibnegger G: Artificial neural networks in laboratory medicine and medical outcome prediction. *Clin Chem Lab Med* 37: 845–853, 1999
- Fonarow GC, Adams KF, Abraham WT, Yancy CW, Boscardin WJ: ADHERE Scientific Advisory Committee, Study Group, and Investigators. Risk stratification for in-hospital mortality in acutely decompensated heart failure: Classification and regression tree analysis. *JAMA* 293: 572–580, 2005
- Goldman L, Cook EF, Brand DA, Lee TH, Rouan GW,

- Weisberg MC, Acampora D, Stasiulewicz C, Walshon J, Terranova G: A computer protocol to predict myocardial infarction in emergency department patients with chest pain. *N Engl J Med* 318: 797–803, 1988
32. Lemon SC, Roy J, Clark MA, Friedmann PD, Rakowski W: Classification and regression tree analysis in public health: Methodological review and comparison with logistic regression. *Ann Behav Med* 26: 172–181, 2003
 33. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, El-Hajj Fuleihan G, Josse RG, Lips P, Morales-Torres J; IOF Committee of Scientific Advisors (CSA) Nutrition Working Group: Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos Int* 20: 1807–1820, 2009
 34. Bikle DD: Vitamin D insufficiency/deficiency in gastrointestinal disorders. *J Bone Miner Res* 22[Suppl 2]: V50–V54, 2007
 35. Grymonprez A, Proesmans W, Van Dyck M, Jans I, Goos G, Bouillon R: Vitamin D metabolites in childhood nephrotic syndrome. *Pediatr Nephrol* 9: 278–281, 1995
 36. Koenig KG, Lindberg JS, Zerwekh JE, Padalino PK, Cushner HM, Copley JB: Free and total 1,25-dihydroxyvitamin D levels in subjects with renal disease. *Kidney Int* 41: 161–165, 1992
 37. Al-Aly Z, Qazi RA, González EA, Zeringue A, Martin KJ: Changes in serum 25-hydroxyvitamin D and plasma intact PTH levels following treatment with ergocalciferol in patients with CKD. *Am J Kidney Dis* 50: 59–68, 2007
 38. Zisman AL, Hristova M, Ho LT, Sprague SM: Impact of ergocalciferol treatment of vitamin D deficiency on serum parathyroid hormone concentrations in chronic kidney disease. *Am J Nephrol* 27: 36–43, 2007
 39. Buccianti G, Bianchi ML, Valenti G, Lorenz M, Cresseri D: Effects of calcifediol treatment on the progression of renal osteodystrophy during continuous ambulatory peritoneal dialysis. *Nephron* 56: 353–356, 1990
 40. Jean G, Terrat JC, Vanel T, Hurot JM, Lorriaux C, Mayor B, Chazot C: Evidence for persistent vitamin D 1-alpha-hydroxylation in hemodialysis patients: Evolution of serum 1,25-dihydroxycholecalciferol after 6 months of 25-hydroxycholecalciferol treatment. *Nephron Clin Pract* 110: c58–c65, 2008
 41. Kalantar-Zadeh K, Kuwae N, Regidor DL, Kovesdy CP, Kilpatrick RD, Shinaberger CS, McAllister CJ, Budoff MJ, Salusky IB, Kopple JD: Survival predictability of time-varying indicators of bone disease in maintenance hemodialysis patients. *Kidney Int* 70: 771–780, 2006
 42. Kovesdy CP, Ahmadzadeh S, Anderson JE, Kalantar-Zadeh K: Association of activated vitamin D treatment and mortality in chronic kidney disease. *Arch Intern Med* 168: 397–403, 2008
 43. Melamed ML, Eustace JA, Plantinga L, Jaar BG, Fink NE, Coresh J, Klag MJ, Powe NR: Changes in serum calcium, phosphate, and PTH and the risk of death in incident dialysis patients: A longitudinal study. *Kidney Int* 70: 351–357, 2006
 44. Naves-Díaz M, Alvarez-Hernández D, Passlick-Deetjen J, Guinsburg A, Marelli C, Rodriguez-Puyol D, Cannata-Andía JB: Oral active vitamin D is associated with improved survival in hemodialysis patients. *Kidney Int* 74: 1070–1078, 2008
 45. Shoben A, Rudser K, De Boer I, Young B, Kestenbaum B: Association of oral calcitriol with improved survival in nondialyzed CKD. *J Am Soc Nephrol* 19: 1613–1619, 2008
 46. Teng M, Wolf M, Lowrie E, Ofsthun N, Lazarus JM, Thadhani R: Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. *N Engl J Med* 349: 446–456, 2003
 47. Teng M, Wolf M, Ofsthun MN, Lazarus JM, Hernán MA, Camargo CA Jr, Thadhani R: Activated injectable vitamin D and hemodialysis survival: A historical cohort study. *J Am Soc Nephrol* 16: 1115–1125, 2005
 48. Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, Hewison M: Extrarenal expression of 25-hydroxyvitamin D(3)-1 alpha-hydroxylase. *J Clin Endocrinol Metab* 86: 888–894, 2001
 49. Lee PS, Sampath K, Karumanchi SA, Tamez H, Bhan I, Isakova T, Gutierrez OM, Wolf M, Chang Y, Stossel TP, Thadhani R: Plasma gelsolin and circulating actin correlate with hemodialysis mortality. *J Am Soc Nephrol* 20: 1140–1148, 2009
 50. DIVINE: Dialysis Infection and Vitamin D in New England. Available online at <http://www.clinicaltrials.gov/ct2/show/NCT00892099>. Accessed January 5, 2010.
 51. Cook NR: Statistical evaluation of prognostic versus diagnostic models: Beyond the ROC curve. *Clin Chem* 54: 17–23, 2008