CJASN ePress. Published on January 23, 2014 as doi: 10.2215/CJN.05450513

Article

Biomarkers of Vascular Calcification and Mortality in Patients with ESRD

Julia J. Scialla, W.H. Linda Kao,†‡ Ciprian Crainiceanu,§ Stephen M. Sozio,‡ Pooja C. Oberai,† Tariq Shafi,‡ Josef Coresh,† Neil R. Powe,‖ Laura C. Plantinga,¶ Bernard G. Jaar,‡** and Rulan S. Parekh†‡††*

Abstract

Background Vascular calcification is common among patients undergoing dialysis and is associated with mortality. Factors such as osteoprotegerin (OPG), osteopontin (OPN), bone morphogenic protein-7 (BMP-7), and fetuin-A are involved in vascular calcification.

Design, setting, participants, & measurements OPG, OPN, BMP-7, and fetuin-A were measured in blood samples from 602 incident dialysis patients recruited from United States dialysis centers between 1995 and 1998 as part of the Choices for Healthy Outcomes In Caring for ESRD Study. Their association with all-cause and cardiovascular mortality were assessed using Cox proportional hazards models adjusted for demographic characteristics, comorbidity, serum phosphate, and calcium. An interaction with diabetes was tested because of its known association with vascular calcification. Predictive accuracy of selected biomarkers was explored by C-statistics in nested models with training and validation subcohorts.

Results Higher OPG and lower fetuin-A levels were associated with higher mortality over up to 13 years of follow-up (median, 3.4 years). The adjusted hazard ratios (HR) for highest versus lowest tertile were 1.49 (95% confidence interval [95% CI], 1.08 to 2.06) for OPG and 0.69 (95% CI, 0.52 to 0.92) for fetuin-A. In stratified models, the highest tertile of OPG was associated with higher mortality among patients without diabetes (HR, 2.42; 95% CI, 1.35 to 4.34), but not patients with diabetes (HR, 1.26; 95% CI, 0.82 to 1.93; *P* for interaction=0.001). In terms of cardiovascular mortality, higher fetuin-A was associated with lower risk (HR, 0.85 per 0.1 g/L: 95% CI, 0.75 to 0.96). In patients without diabetes, higher OPG was associated with greater risk (HR for highest versus lowest tertile, 2.91; 95% CI, 1.06 to 7.99), but not in patients with diabetes or overall. OPN and BMP-7 were not independently associated with outcomes overall. The addition of OPG and fetuin-A did not significantly improve predictive accuracy of mortality.

Conclusions OPG and fetuin-A may be risk factors for all-cause and cardiovascular mortality in patients undergoing dialysis, but do not improve risk prediction.

Clin J Am Soc Nephrol 9: ●●●–●●●, 2014. doi: 10.2215/CJN.05450513

*Department of Medicine, University of Miami, Miami, Florida; †Department of Epidemiology, §Department of Biostatistics, and ‡Department of Medicine, Johns Hopkins University, Baltimore, Maryland; ‖Department of Medicine, San Francisco General Hospital and University of California San Francisco, San Francisco, California; ¶Department of Epidemiology, Emory University, Atlanta, Georgia; **Nephrology Center of Maryland, Baltimore, Maryland; and ††Hospital for Sick Children, University Health Network and University of Toronto, Toronto, Ontario, Canada

Correspondence: Dr. Rulan S. Parekh, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada. Email: Rulan.parekh@sickkids.ca

Introduction

The mortality rate among patients with ESRD who are undergoing dialysis is approximately seven times greater than for similar individuals in the general population and is largely attributed to cardiovascular causes. Although survival has improved modestly over the past decade, the annual adjusted mortality rate of 185.8 per 1000 patient-years remains high (1). Detailed investigations into novel uremia-related risk factors that contribute to excess cardiovascular mortality may identify promising therapeutic targets in ESRD.

Decades of clinical observations have noted deposition of extraosseous calcium in patients with ESRD (2,3). Medial calcification of large vessels, intimal calcification of coronary arteries, and calcification of the cardiac valves are each associated with higher mortality in patients with ESRD (4). They may be initiated by the uremic milieu, as well as comorbid factors, such as diabetes, inflammation, and hyperphosphatemia (5,6). Animal models have identified osteoprotegerin (OPG), osteopontin (OPN), bone morphogenic protein-7 (BMP-7), and fetuin-A as factors that may regulate calcification in the vasculature (7–13).

Fetuin-A is secreted by hepatocytes into the circulation, where it forms soluble complexes with calcium and phosphate (11,14). OPG, OPN, and BMP-7 are produced by a variety of cell types, including endothelial and vascular smooth muscle cells, with increased expression in the setting of active calcification (12,14,15). Numerous clinical studies have demonstrated associations between these biomarkers and measures of vascular calcification and stiffness (16–22). In this study, we evaluated the association between selected calcification biomarkers and long-term mortality in a large, prospective cohort of incident dialysis patients. Additionally, we assessed whether the associations were independent of established calcification stimuli, such as abnormal phosphate metabolism, diabetes, and inflammation.

Materials and Methods

Study Population

Participants were from the Choices for Healthy Outcomes In Caring for ESRD (CHOICE) study. CHOICE enrolled 1041 participants from United States dialysis centers in a multicenter, prospective cohort study between 1995 and 1998. Included in the study were 602 participants with blood samples available. The study was approved by the Institutional Review Board at Johns Hopkins University School of Medicine, and all participants provided written informed consent.

Data Collection and Measurement

Calcification biomarkers were measured within 6 months of study enrollment using enzyme immunoassays. Samples were collected before dialysis treatment, processed, and stored in −80°C freezers. OPG (coefficient of variation [CV]<10%; Alpco Diagnostics), fetuin-A (CV <15%; Epitope Diagnostics), and BMP-7 (CV<10%; R&D Systems) were measured in serum, and OPN (CV<10%; R&D Systems) was measured in plasma. Routine laboratory measures were averaged from all values available in the first 3 months of dialysis. Corrected serum calcium was calculated (corrected calcium [mg/dl]=serum calcium [mg/dl]+0.8 [4−serum albumin [g/dl]). IL-6, C-reactive protein, and C-terminal fibroblast growth factor-23 (FGF-23; Immutopics, San Clemente, CA) were measured within 6 months of enrollment.

Baseline comorbid conditions were abstracted from predialysis medical records, and the Index of Co-Existent Disease (ICED) was calculated (23). Deaths were ascertained through March 31, 2009, by linkage with the US Renal Data System. Cause of death was ascertained through December 31, 2004, from the National Death Index, with chart review to confirm cause of death when possible, as previously described (24). A cardiovascular cause of death included coronary artery disease, cerebrovascular disease, peripheral vascular disease, and abdominal aortic aneurysms.

Statistical Analyses

Baseline characteristics of the study population were described and compared between patients with diabetes and patients without diabetes. Laboratory characteristics were compared across tertiles of each calcification biomarker using linear regression for continuous variables or a Pearson's chi-squared test for categorical variables. Multiple imputation by chained equations was used to impute missing covariates (five cycles) before multivariable models were built (25). The following covariates were imputed using all other covariates as predictors: history of cardiovascular disease (n=2; <1%), history of diabetes (n=1; <1%), body mass index (n=33; 5%), index of coexistent disease (n=1; <1%), serum phosphate (n=3; <1%), serum calcium (n=4; <1%), serum albumin (n=4; <1%), C-reactive protein (n=35; 6%), IL-6 (n=37; 6%), and FGF-23 (n=91; 15%).

Unadjusted survival was compared across tertiles of each biomarker using the Kaplan–Meier method and log-rank tests. To adjust for confounding, the relative hazard of mortality by levels of calcification biomarkers (continuous and tertiles) was modeled using Cox proportional hazards models stratified by clinic cluster. Survival time was from dialysis initiation to death, with entry when calcification biomarkers were measured. Participants were censored at transplantation (n=140) or last follow-up (n=39). Our main model (model 1) was adjusted for demographic factors, comorbidity (including ICED, diabetes, and prevalent cardiovascular disease at dialysis initiation), body mass index, serum phosphate, and corrected serum calcium. Model 2 was additionally adjusted for inflammatory markers (serum albumin, log [C-reactive protein] and log [IL-6]) and log (FGF-23). Interactions were tested between calcification biomarkers and sex, race, diabetic status, prior cardiovascular disease, high versus normal serum phosphate (>5.5 mg/dl versus ≤5.5 mg/dl) and the presence of inflammation (defined as serum albumin<3.6 g/dl, C-reactive protein≥10 mg/L, or IL-6≥3.09 pg/ml, as previously described [24]) using stratified models and interaction terms in model 1. Restricted cubic splines were fit to explore nonlinear relationships between levels of calcification biomarkers and all-cause mortality after multivariable adjustment. If nonlinearity was suspected, piecewise linear splines were fit to test for significant changes in slope at defined knots.

We assessed cardiovascular mortality in secondary analyses. Because of fewer events, these models were not additionally adjusted for inflammatory markers or FGF-23. In sensitivity analyses, we repeated analyses using unimputed data and model-wise deletion to handle missing data.

To evaluate predictive accuracy, we evaluated C-statistics in models incorporating OPG and fetuin-A compared with base models. We used unimputed data among those with complete covariate information (*i.e.*, complete case analysis) to ensure that nested models were comparable (n=428). We randomly assigned half of the diabetic patients and half of the nondiabetic patients to training (n=213) or validation (n=215) datasets. In the training set, we fit models of all-cause mortality with and without OPG as a continuous linear predictor and fetuin-A as a piecewise linear spline. We calculated a C-statistic for each model in the validation cohort and tested for differences in C-statistic across nested models (26).

All analyses were performed using Stata software, special edition 11.0 (Stata Corp., College Station, Texas) and a two-sided α value of 0.05.

Results

The majority of the study population was treated with hemodialysis (95%) (Table 1). The median OPG was 10.9 (interquartile range [IQR], 8.0–15.3) pmol/L, the median fetuin-A was 0.49 (IQR, 0.40–0.60) g/L, the median OPN was 150.6 (IQR, 94.8–229.1) ng/ml, and the median BMP-7 was 13.7 (IQR, 10.3–19.2) pg/ml. Fifty-seven percent of the study population was diabetic. Diabetic participants were older, were more likely to be overweight or obese, had greater cardiovascular comorbidity, and had higher levels of OPG and lower levels of fetuin-A (Table 1).

Unadjusted levels of calcium, phosphate, inflammatory markers, and FGF-23 are presented by tertiles of calcification biomarkers in Table 2. Higher levels of OPG were associated with lower serum phosphate. Higher levels of OPG and OPN and lower levels of fetuin-A were associated with increased indices of inflammation (*i.e.*, lower serum albumin and higher C-reactive protein and IL-6). Patterns of univariate associations with the selected

Table 1. Baseline characteristics of study population overall and compared across diabetic status

Clinical Characteristic	Overall (*n*=602)	Diabetic[a] (*n*=340)	Nondiabetic (*n*=261)	*P* Value[b]
Age (yr)	57.8±14.9	59.2±12.3	55.9±17.6	0.01
Sex				0.25
Male	320 (53.2)	174 (51.2)	146 (55.9)	
Female	282 (46.8)	166 (48.8)	115 (44.1)	
Race				0.04
White	357 (59.3)	193 (56.8)	163 (62.5)	
Black	211 (35.1)	121 (35.6)	90 (34.5)	
Other	34 (5.7)	26 (7.7)	8 (3.1)	
Cause of ESRD				<0.001
Diabetes	293 (48.7)	293 (86.2)	—	
Hypertension	106 (17.6)	21 (6.2)	85 (32.6)	
GN	94 (15.6)	11 (3.2)	83 (31.8)	
Other	109 (18.1)	15 (4.4)	93 (35.6)	
Dialysis modality				0.91
Hemodialysis	569 (94.5)	321 (94.4)	247 (94.6)	
Peritoneal dialysis	33 (5.5)	19 (5.6)	14 (5.4)	
Comorbid disease				
Diabetes	340 (56.5)	—	—	
Cardiovascular disease	253 (42.0)	182 (53.7)	71 (27.2)	<0.001
Congestive heart failure	292 (48.5)	201 (59.1)	91 (34.9)	<0.001
Prior myocardial infarction	143 (23.8)	102 (30.0)	41 (15.7)	<0.001
Smoking				0.04
Never	241 (40.0)	138 (40.6)	102 (39.1)	
Former	246 (40.9)	146 (42.9)	100 (38.3)	
Current	97 (16.1)	43 (12.6)	54 (20.7)	
Body mass index				<0.001
Underweight (≤18.0 kg/m^2)	23 (3.8)	12 (3.5)	11 (4.2)	
Ideal weight (18.1–25.0 kg/m^2)	231 (38.4)	107 (31.5)	124 (47.5)	
Overweight (25.1–30.0 kg/m^2)	161 (26.7)	96 (28.2)	65 (24.9)	
Obese (>30 kg/m^2)	154 (25.6)	106 (31.2)	47 (18.0)	
Calcification biomarkers				
Osteoprotegerin (pmol/L)[c]	10.9 (8.0–15.3)	12.2 (9.4–15.9)	9.4 (6.7–13.6)	<0.001
Fetuin-A (g/L)[c]	0.49 (0.40–0.60)	0.46 (0.38–0.57)	0.53 (0.44–0.63)	<0.001
Bone morphogenic protein-7 (pg/ml)[c]	13.7 (10.3–19.2)	13.5 (10.0–19.9)	13.8 (10.5–18.8)	0.57
Osteopontin (ng/ml) [c]	150.6 (94.8–229.1)	144.9 (96.9–216.2)	159.6 (91.4–254.3)	0.19

Unless otherwise noted, values are expressed as the mean±SD or number (percentage) of patients.
[a]Diabetic status was unknown in one participant.
[b]*P* value compares patients with diabetes versus patients without diabetes using ANOVA or Kruskal–Wallis test for continuous variables and chi-squared test for categorical variables.
[c]Presented as median (interquartile range)

laboratory measures were similar when stratified by diabetic status (data not shown).

A total of 423 patients died over a period of up to 13.3 years (median follow-up of 3.4 years). Median survival in the cohort was 4.3 years, but was shorter in patients with diabetes (4 years) than in those without (5 years) (Figure 1). Median follow-up was shorter than median survival, because of early censoring among patients who received kidney transplants (*n*=140). Higher tertiles of OPG were associated with decreased survival, with median survival durations of 6.3, 4.3, and 3.7 years in tertiles 1, 2, and 3, respectively ($P<0.001$) (Supplemental Figure 1A). Lower tertiles of fetuin-A were associated with decreased survival overall, with median survival durations of 3.5, 4.3, and 5.6 years in tertiles 1, 2, and 3 ($P<0.001$) (Supplemental Figure 1B). Higher tertiles of OPN were associated with decreased survival, with median survival durations of 5.4, 3.9, and 3.9 years in tertiles 1, 2, and 3 ($P=0.02$) (Supplemental Figure 1C). BMP-7 was not associated with survival ($P=0.23$).

Adjusted hazard ratios (HRs) for mortality by tertiles of calcification biomarkers are presented in Table 3. Overall, higher OPG and lower fetuin-A were associated with higher hazard of mortality after multivariable adjustment ($P=0.01$ and 0.01, respectively). Additional adjustment for inflammatory markers (*i.e.*, serum albumin, C-reactive protein, and IL-6) and FGF-23 attenuated these associations. Tertiles of OPN and BMP-7 were not associated with hazard of mortality.

The associations between serum OPG levels and mortality were significantly modified by diabetes (*P* for interaction=0.001) (Supplemental Figure 2). Among patients

Table 2. Selected laboratory variables by tertiles of calcification biomarkers

Variable	Corrected Calcium (mg/dl)	Phosphate (mg/dl)	Fibroblast Growth Factor-23 (RU/ml)	Serum Albumin (g/dl)	C-Reactive Protein (mg/l)	IL-6 (pg/ml)	Osteoprotegerin (pmol/l)	Fetuin-A (g/l)	Bone Morphogenic Protein-7 (pg/ml)	Osteopontin (ng/ml)
Osteoprotegerin[a]										
Tertile 1	9.34±0.67	5.48±1.35	1506 (685–4183)	3.72±0.39	3.8 (1.6–10.5)	3.7 (2.4–6.7)	6.9 (5.5–8.0)	0.51 (0.42–0.59)	13.0 (10.3–18.8)	128.8 (71.3–203.1)
Tertile 2	9.42±0.59	5.38±1.23	1794 (1006–6074)	3.65±0.35	4.3 (2.0–9.7)	4.2 (2.7–7.4)	10.9 (9.9–12.2)	0.50 (0.41–0.62)	14.1 (10.7–19.6)	151.1 (104.0–228.4)
Tertile 3	9.45±0.58	5.10±1.24	2043 (915–6089)	3.56±0.29	5.1 (2.2–13.5)	5.3 (3.1–9.7)	17.0 (15.3–21.1)	0.47 (0.37–0.59)	14.1 (10.1–19.0)	167.9 (111.7–253.7)
P value[b]	0.11	0.003	0.17	<0.001	0.02	<0.001	–	0.09	0.92	0.01
Fetuin-A[a]										
Tertile 1	9.42±0.59	5.23±1.24	1677 (1001–4788)	3.57±0.32	5.3 (2.7–16.8)	5.3 (3.2–11.6)	11.8 (8.7–16.1)	0.37 (0.33–0.40)	13.9 (9.8–20.5)	158.9 (101.0–232.4)
Tertile 2	9.39±0.61	5.30±1.35	1651 (757–5375)	3.65±0.32	4.1 (1.5–12.5)	4.0 (2.6–7.4)	10.1 (7.6–13.9)	0.49 (0.46–0.52)	14.6 (11.4–21.5)	151.8 (107.4–254.4)
Tertile 3	9.40±0.66	5.42±1.27	1584 (839–6372)	3.71±0.39	3.4 (1.6–7.4)	3.7 (2.4–6.9)	10.9 (7.6–15.3)	0.65 (0.60–0.76)	13.0 (9.7–17.4)	129.5 (73.8–216.2)
P value[b]	0.78	0.15	0.75	<0.001	<0.001	<0.001	0.02	–	0.28	0.01
Bone morphogenic protein-7[a]										
Tertile 1	9.42±0.62	5.15±1.17	1507 (829–3876)	3.67±0.35	4.2 (1.7–11.4)	4.0 (2.8–7.8)	10.4 (7.8–14.1)	0.48 (0.38–0.60)	8.6 (6.4–10.3)	150.6 (98.6–224.8)
Tertile 2	9.42±0.65	5.45±1.37	1552 (784–5888)	3.67±0.35	4.2 (1.8–10.5)	4.1 (2.5–7.3)	10.7 (7.8–15.1)	0.49 (0.40–0.58)	13.7 (12.5–15.0)	131.0 (73.0–211.1)
Tertile 3	9.40±0.60	5.42±1.33	2057 (936–7073)	3.63±0.30	4.4 (1.6–11.2)	4.6 (2.5–8.4)	10.7 (7.6–15.3)	0.47 (0.40–0.57)	23.8 (19.3–29.2)	160.5 (111.1–259.1)
P value[b]	0.82	0.11	0.04	0.20	0.89	0.90	0.51	0.55	–	0.10
Osteopontin[a]										
Tertile 1	9.35±0.69	5.23±1.21	1477 (773–3276)	3.74±0.32	3.8 (1.7–6.8)	3.8 (2.5–6.5)	9.4 (7.0–13.0)	0.50 (0.42–0.61)	13.1 (9.8–18.2)	67.6 (33.3–94.8)
Tertile 2	9.39±0.57	5.33±1.30	1547 (690–4468)	3.64±0.34	4.4 (2.0–12.3)	4.4 (2.7–7.3)	11.2 (8.4–15.3)	0.47 (0.39–0.56)	14.1 (10.4–19.6)	150.6 (135.0–172.1)
Tertile 3	9.47±0.59	5.36±1.39	2200 (1030–7779)	3.59±0.33	4.3 (1.9–13.3)	5.3 (3.1–10.2)	11.8 (8.0–15.6)	0.47 (0.38–0.58)	14.3 (10.5–20.0)	289.0 (229.1–381.9)
P value[b]	0.08	0.38	0.003	<0.001	0.26	0.02	0.004	0.03	0.41	–

Values are the mean±SD or median (interquartile range).

[a]Sample size varies by biomarker because of availability of blood samples: osteoprotegerin, *n*=580; fetuin-A, *n*=579; bone morphogenic protein-7, *n*=498; osteopontin, *n*=510.

[b]*P* trend from linear regression. Outcome variables were log transformed before testing in the case of skewed variables (fibroblast growth factor-23, C-reactive protein, IL-6, osteoprotegerin, fetuin-A, bone morphogenic protein-7, and osteopontin).

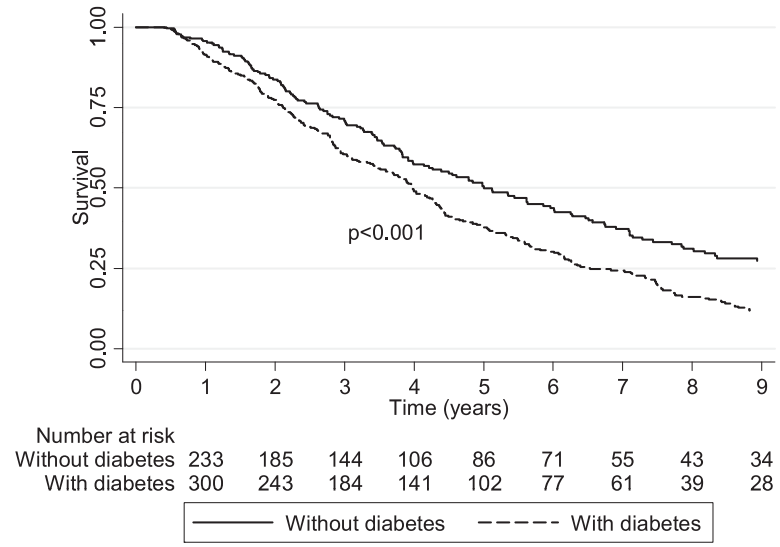

Figure 1. | **Lower cumulative survival among participants with diabetes compared to those without**. Unadjusted cumulative survival in overall study population among those with diabetes (dashed line) and those without diabetes (solid line). *P* value represents a test of different survival between those with and without diabetes by the log-rank test.

with diabetes, we did not observe an association between OPG levels and mortality; however, among patients without diabetes, higher tertiles of OPG were associated with a graded risk of mortality, with a hazard that was 2.42 times higher in the highest tertile than in the lowest (P=0.003) (Table 3). This association was not attenuated after additional adjustment for inflammatory markers and FGF-23 (HR, 2.37; 95% confidence interval [95% CI], 1.25 to 4.49 for the highest tertile compared with the lowest). The association between fetuin-A and mortality was similar among patients with diabetes and those without diabetes (P for interaction=0.65). Among patients without diabetes, OPN demonstrated higher risk of mortality in the highest tertile compared with the lowest (P=0.01) and remained significant with adjustment for inflammation and FGF-23 (P=0.05). A similar pattern was not observed among patients with diabetes, but the interaction between these calcification biomarkers and diabetes was not statistically significant. The association of both OPG and fetuin-A was similar across subgroups defined by sex, race, history of prior cardiovascular disease, serum phosphate concentrations, and biochemical evidence of inflammation (Supplemental Figure 3).

In exploratory models, we did not observe strong evidence for nonlinear relationships between the levels of OPG, OPN, or BMP-7 and the hazard of mortality using restricted cubic splines, but levels of fetuin-A demonstrated a threshold effect with the hazard of mortality (Supplemental Figure 4). Using piecewise linear splines in fully adjusted models (model 2), we found significant nonlinearity (P=0.04), with an increasing risk among those with levels <0.50 g/L (P=0.01), but no independent relationship between fetuin-A levels and mortality if fetuin-A was >0.50 g/L (P=0.51).

During a period of up to 9.3 years (median, 3.4 years), there were 186 cardiovascular deaths and 195 deaths due to noncardiovascular causes. In unadjusted analyses, the hazard of death from cardiovascular disease in the highest tertile of OPG was 2.87 times higher than in the lowest tertile (95% CI, 1.90 to 4.35; P<0.001). By tertiles of fetuin-A, the unadjusted hazard of death from cardiovascular disease was 52% lower (HR, 0.48; 95% CI, 0.32 to 0.71; P<0.001). Results attenuated after multivariable adjustment but demonstrated a pattern similar to that of all-cause mortality (Table 4). Diabetes modified the association of cardiovascular mortality with OPG (P for interaction=0.001), but not fetuin-A (P for interaction=0.09). Results for all-cause and cardiovascular mortality were similar in sensitivity analyses using unimputed data (Supplemental Tables 1–3).

We explored the predictive accuracy of OPG and fetuin-A. Both OPG and fetuin-A within the lower range were independently associated with all-cause mortality after multivariable adjustment (model 1) among participants in the training set (HR, 1.25 [95% CI, 1.07 to 1.46] per 5 pmol/L higher OPG; HR, 0.76 [95% CI, 0.60 to 0.97] per 0.1 g/L higher fetuin-A when <0.5 g/L). However, their inclusion did not significantly improve predictive accuracy, as quantified by the C-statistic, compared with multivariable models or models that included inflammatory markers and FGF-23 (Table 5).

Discussion

We observed that levels of calcification biomarkers are associated with mortality in persons with ESRD. Specifically, higher levels of OPG were associated with significantly greater risk of all-cause and cardiovascular mortality; however, this was most striking in participants without diabetes. OPN demonstrated a similar pattern, with higher risk for all-cause mortality observed among those with the highest levels, but only among patients without diabetes. In contrast, we observed that lower levels of fetuin-A were associated with greater risk of all-cause and cardiovascular mortality and a threshold effect, with higher risk for those with levels

Table 3. Adjusted hazard ratios (95% confidence intervals) of all-cause mortality by levels of calcification biomarkers and stratified by diabetic status

Variable	Multivariable Adjusted[a]			Additionally Adjusted for Inflammation and Fibroblast Growth Factor-23[a]		
	All (*n*=602)	Diabetic[b] (*n*=341)	Nondiabetic (*n*=261)	All	Diabetic	Nondiabetic
Osteoprotegerin (*n*=580)						
Tertile 1	Reference	Reference	Reference	Reference	Reference	Reference
Tertile 2	1.34 (1.00 to 1.80)	1.19 (0.79 to 1.78)	1.39 (0.81 to 2.40)	1.26 (0.93 to 1.73)	1.06 (0.70 to 1.62)	1.33 (0.73 to 2.43)
Tertile 3	1.49 (1.08 to 2.06)[c]	1.26 (0.82 to 1.93)	2.42 (1.35 to 4.34)[c]	1.27 (0.89 to 1.80)	1.03 (0.65 to 1.63)	2.37 (1.25 to 4.49)[c]
Continuous (per 5 pmol/L)	1.16 (1.04 to 1.29)[c]	1.08 (0.93 to 1.24)[d]	1.46 (1.16 to 1.83)[c,d]	1.07 (0.95 to 1.20)	1.00 (0.86 to 1.16)[d]	1.43 (1.12 to 1.82)[c,d]
Fetuin-A (*n*=579)						
Tertile 1	Reference	Reference	Reference	Reference	Reference	Reference
Tertile 2	0.93 (0.72 to 1.22)	1.09 (0.78 to 1.53)	0.77 (0.47 to 1.29)	0.98 (0.75 to 1.28)	1.01 (0.72 to 1.42)	0.92 (0.53 to 1.59)
Tertile 3	0.69 (0.52 to 0.92)[c]	0.64 (0.44 to 0.93)[c]	0.61 (0.36 to 1.01)	0.77 (0.57 to 1.04)	0.63 (0.42 to 0.92)[c]	0.82 (0.46 to 1.44)
Continuous (per 0.1 g/L)	0.90 (0.83 to 0.98)[c]	0.88 (0.79 to 0.98)[c]	0.89 (0.77 to 1.02)	0.94 (0.87 to 1.02)	0.89 (0.80 to 1.00)[c]	0.98 (0.84 to 1.15)
Osteopontin (*n*=510)						
Tertile 1	Reference	Reference	Reference	Reference	Reference	Reference
Tertile 2	1.21 (0.90 to 1.62)	1.28 (0.86 to 1.91)	1.10 (0.62 to 1.97)	1.11 (0.82 to 1.51)	1.18 (0.78 to 1.78)	0.97 (0.53 to 1.78)
Tertile 3	1.21 (0.89 to 1.63)	1.02 (0.68 to 1.53)	2.11 (1.17 to 3.81)[c]	1.06 (0.77 to 1.45)	0.80 (0.52 to 1.24)	1.94 (1.00 to 3.74)[c]
Continuous (per 50 ng/ml)	1.01 (0.96 to 1.06)	0.99 (0.93 to 1.07)	1.09 (0.99 to 1.21)	0.98 (0.93 to 1.03)	0.95 (0.88 to 1.02)	1.08 (0.96 to 1.21)
Bone morphogenic protein-7 (*n*=498)						
Tertile 1	Reference	Reference	Reference	Reference	Reference	Reference
Tertile 2	0.99 (0.74 to 1.32)	1.04 (0.71 to 1.53)	0.82 (0.47 to 1.44)	0.99 (0.74 to 1.33)	1.02 (0.69 to 1.52)	0.75 (0.41 to 1.34)
Tertile 3	1.08 (0.82 to 1.44)	1.10 (0.75 to 1.60)	1.65 (0.92 to 2.98)	1.01 (0.76 to 1.35)	1.06 (0.71 to 1.57)	1.70 (0.91 to 3.18)
Continuous (per ln)[e]	1.10 (0.91 to 1.33)	1.10 (0.85 to 1.42)	1.27 (0.86 to 1.88)	1.01 (0.83 to 1.24)	1.03 (0.78 to 1.36)	1.26 (0.80 to 1.98)

[a]Multivariable model adjusted for age, sex, race, index of coexistent disease, diabetes, cardiovascular disease, body mass index, serum phosphate, and corrected serum calcium; additionally adjusted model also adjusted for serum albumin, log IL-6, log C-reactive protein, and log fibroblast growth factor-23.
[b]Diabetes status was imputed for one participant.
[c]$P<0.05$.
[d]P for interaction<0.01 from model using biomarker as a continuous variable.
[e]Natural log transformed before continuous modeling because of highly skewed distribution.

Table 4. Hazard ratios (95% confidence intervals) of cardiovascular mortality by tertiles of osteoprotegerin and fetuin-A and stratified by diabetes

Variable	Unadjusted				Adjusted[a]			
	All (*n*=580)	Diabetic[b] (*n*=329)	Nondiabetic (*n*=251)	*P* Value for Interaction[c]	All	Diabetic	Nondiabetic	*P* Value for Interaction[c]
Osteoprotegerin (*n*=580)								
Tertile 1	Reference	Reference	Reference		Reference	Reference	Reference	
Tertile 2	1.80 (1.17 to 2.77)[d]	1.08 (0.64 to 1.84)	3.52 (1.46 to 8.48)[d]		1.24 (0.78 to 1.96)	0.94 (0.53 to 1.65)	2.06 (0.76 to 5.58)	
Tertile 3	2.87 (1.90 to 4.35)[d]	1.34 (0.80 to 2.26)	7.43 (3.29 to 16.79)[d]		1.59 (0.99 to 2.55)	1.09 (0.62 to 1.93)	2.91 (1.06 to 7.99)[d]	
Continuous (per 5 pmol/L)	1.35 (1.19 to 1.53)[d]	1.02 (0.86 to 1.21)	1.99 (1.55 to 2.56)[d]	<0.01	1.13 (0.96 to 1.32)	0.96 (0.79 to 1.18)	1.38 (0.97 to 1.98)	<0.01
Fetuin-A (*n*=579)								
Tertile 1	Reference	Reference	Reference		Reference	Reference	Reference	
Tertile 2	0.66 (0.46 to 0.95)[d]	1.06 (0.68 to 1.65)	0.30 (0.14 to 0.62)[d]		0.86 (0.59 to 1.27)	1.19 (0.76 to 1.88)	0.43 (0.18 to 1.01)	
Tertile 3	0.48 (0.32 to 0.71)[d]	0.68 (0.41 to 1.13)	0.34 (0.17 to 0.69)[d]		0.69 (0.45 to 1.05)	0.74 (0.43 to 1.27)	0.42 (0.18 to 0.98)[d]	
Continuous (per 0.1 g/L)	0.76 (0.67 to 0.86)[d]	0.86 (0.74 to 0.99)[d]	0.69 (0.54 to 0.87)[d]	0.10	0.85 (0.75 to 0.96)[d]	0.89 (0.77 to 1.04)	0.71 (0.55 to 0.93)[d]	0.09

[a]Adjusted for age, sex, race, index of coexistent disease, diabetes, cardiovascular disease, body mass index, serum phosphate, and corrected serum calcium.
[b]Diabetes status was imputed for one participant.
[c]*P* value for interaction from model using biomarker as a continuous variable.
[d]$P<0.05$.

Table 5. Predictive accuracy (C-statistics and 95% confidence intervals) of all-cause mortality using nested models stratified by diabetes

Model	Overall (n=215)[a]	Diabetes (n=122)[a]	No Diabetes (n=93)[a]
Demographic model[b]	0.70 (0.65 to 0.75)	0.64 (0.58 to 0.71)	0.78 (0.71 to 0.85)
Demographic model + osteoprotegerin/fetuin-A[c]	0.69 (0.64 to 0.74)	0.64 (0.57 to 0.71)	0.76 (0.69 to 0.82)
P value for difference	0.68	0.97	0.30
Model 1	0.72 (0.67 to 0.76)	0.70 (0.64 to 0.76)	0.75 (0.67 to 0.82)
Model 1+ osteoprotegerin/fetuin-A	0.72 (0.68 to 0.77)	0.70 (0.64 to 0.76)	0.75 (0.68 to 0.81)
P value for difference	0.47	0.70	0.91
Model 2	0.74 (0.70 to 0.78)	0.71 (0.65 to 0.77)	0.79 (0.73 to 0.85)
Model 2 + osteoprotegerin/fetuin-A	0.74 (0.69 to 0.78)	0.71 (0.65 to 0.77)	0.78 (0.72 to 0.84)
P value for difference	0.34	0.93	0.35

[a]The n value is sample size of validation dataset.
[b]Demographic model is adjusted for age, sex, and race (black/other versus white); model 1 is adjusted for all variables in the demographic model plus history of cardiovascular disease, history of diabetes, body mass index, index of coexistent disease (mild, moderate or severe), serum phosphate, and corrected serum calcium; model 2 is adjusted for all variables in model 1 plus serum albumin, log (C-reactive protein), log (IL-6), and log (fibroblast growth factor-23).
[c]Osteoprotegerin is modeled linearly as a continuous variable, fetuin-A is modeled as a continuous variable using piecewise linear splines with a knot at 0.5 g/L.

<0.5 g/L. The overall pattern we observed in this study is best explained by active vascular production of OPG and OPN and consumption of the circulating calcification inhibitor, fetuin-A, in patients with calcifying vascular disease.

The observed association of higher OPG levels and mortality is consistent with previous reports in dialysis patients (27); however, differences by diabetic status have not been previously reported. OPG is secreted directly from the vascular wall, where it modulates apoptosis, inflammation, and calcium deposition (28). Additionally, its primary role may be in bone, where OPG is secreted by osteoblasts to inhibit differentiation and maturation of neighboring osteoclasts (10,29,30). Higher levels of OPG probably indicate a compensatory increase of OPG locally in the vascular wall to counteract vascular calcium deposition or result from the transition of vascular smooth muscle cells to cells resembling osteoblasts. OPN is also secreted locally in the vascular wall and bone tissue and demonstrated a similar pattern of risk, with the highest levels of this marker associated with higher risk of mortality among patients without diabetes. In aggregate, these results implicate the active process of vascular calcification in the high risk of mortality and cardiovascular disease in patients undergoing dialysis.

Remarkably, the association between higher levels of the calcification biomarkers, OPG and OPN, and higher risk of mortality was dramatically stronger among patients without diabetes than among patients with diabetes. Diabetes is a strong independent stimulus for vascular calcification and is associated with poor outcomes among patients receiving dialysis (1,31). In our study population, cumulative survival was worse among those with than in those without diabetes. It is possible that among a population with a very high prevalence of vascular calcification and poor outcomes, higher levels of calcification biomarkers may be less informative. Additionally, circulating levels of these biomarkers may be influenced by glucose and insulin levels, which further limits their utility in this population (32,33). Associations between OPG and mortality among patients without diabetes were independent of inflammation and FGF-23, which is noteworthy because each is an independent risk factor for mortality in patients undergoing dialysis (34–36). In fact, FGF-23 has been associated with vascular calcification in several studies (37–39), but not consistently (40), and experimental studies do not support a direct role of FGF-23 in promoting calcification (40,41). Prior associations between FGF-23 and calcification may be in part due to its correlation with phosphate and klotho deficiency. It is more likely that the role of FGF-23 leading to higher mortality in dialysis patients is due to the development of left ventricular hypertrophy (42). Additionally, questions still remain on racial differences in FGF-23 and other mineral metabolites, which is beyond the scope of the current manuscript (35).

Although OPG, OPN, and BMP-7 are produced locally in the vascular wall, fetuin-A is produced in the liver. Fetuin-A concentrations are high in calcified vascular lesions, where it is deposited from the circulation in conjunction with calcium and phosphate (14). This alternative physiology probably explains why the pattern of association between fetuin-A and mortality differs from that seen with the other calcification biomarkers. We corroborate, as well as extend, the findings in other cohorts of patients with ESRD that deficiency of fetuin-A is associated with higher all-cause and cardiovascular mortality (43–47). Adjustment for inflammation and FGF-23 weakened the association between fetuin-A levels and mortality, with loss of statistical significance. Fetuin-A is a negative acute phase reactant that is correlated with other inflammatory markers; therefore, these models may have been overadjusted (46). It is plausible that inflammation and fetuin-A are factors in a shared pathophysiologic pathway, whereby chronic inflammation contributes to deficiency of fetuin-A, promoting dystrophic calcification and higher mortality risk (46). Lower fetuin-A levels have not been consistently associated with mortality or cardiovascular disease in populations not undergoing dialysis (16,48–51). In this study, we observed a threshold effect whereby only levels <0.5

g/L were associated with increasing mortality, which may account for apparently conflicting results. Alternatively, differences in assay performance may contribute to the heterogeneity (52).

Despite the independent associations that we observed between OPG, fetuin-A, and mortality, the inclusion of these biomarkers did not improve clinical risk prediction in this study, and our findings do not support immediate application of these calcification biomarkers in risk prediction algorithms. It is important to note that our sample size for model building and internal validation was limited, particularly after stratification by the presence of diabetes, and confidence limits for our C-statistics were wide. Therefore, small effects on predictive capability cannot be excluded. Additionally, predictive accuracy was modest overall, even in models that included inflammation and FGF-23. Despite the negative findings in terms of robust risk prediction, the independent associations with mortality highlight the potential etiologic role of dysregulated calcification in the high mortality of dialysis patients.

This study has many strengths, including a prospective design of incident dialysis patients with complete ascertainment of mortality, classification of cardiovascular mortality using validated methods, and measurement of multiple confounding variables, including adjudicated comorbidity. The study also has several notable limitations. Although we have proposed that variability in the measured biomarkers may be related to the state of calcification in the vasculature, we did not have direct measures of calcification in this study to corroborate this. In fact, the circulating factors measured in this study probably have pleiotropic effects, including roles in apoptosis and inflammation (53), that could account for the observed associations. Furthermore, we cannot determine whether these factors play a causal role in the development of disease or are markers of the underlying pathophysiologic state. Many of the calcification biomarkers, such as OPN, are subject to phosphorylation and other post-translational modifications that affect their biologic activity and that we could not measure (54), and single measurements may not adequately capture their variability over time. In addition, currently available serum assays do not distinguish between free serum fetuin-A and fetuin-A bound in fetuin-mineral complexes, which become more prevalent with advancing CKD (55). Finally, although we found potentially important interactions between the presence of diabetes at baseline and some calcification biomarkers, we did not evaluate the impact of diabetic control on these factors in this study.

Our findings support the role of calcification in excess mortality, particularly cardiovascular mortality, noted among patients with ESRD undergoing dialysis. The association between the calcification biomarkers OPG and OPN may be stronger among dialysis patients without diabetes, underscoring the heterogeneity of the dialysis population. These findings highlight the importance of these novel pathways of calcification in the adverse outcomes of patients receiving dialysis and identify candidate targets for future clinical applications.

Acknowledgments

The authors thank the participants, staff, laboratory, and medical directors from the participating clinics of Dialysis Clinic, Inc., for their contributions to this study.

This work was primarily supported by a grant from Abbvie Laboratories (R.S.P.). Additional support was provided by the National Institutes of Health (NIH) 5KL2RR025006 from the National Center for Research Resources, NIH Roadmap for Medical Research (J.J.S.), NIH National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) K24DK02643 (N.R.P.), 5K01DK067207-05 (W.H.L.K.), 5U01DK070657-03 (W.H.L.K.), 5R01DK072367-03 (W.H.L.K. and R.S.P.), T32DK007732-14 (J.J.S.), K23DK095949 (J.J.S.), and K23DK083514 (T.S.). CHOICE was supported by NIH NIDDK R01DK080123 and R01DK059616, NIH National Heart, Lung, and Blood Institute R01HL62985, and Agency for Healthcare Research and Quality R01HS008365. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIDDK, the NIH, or Abbvie Laboratories.

This work was presented in abstract form at the American Society of Nephrology's Kidney Week in Philadelphia, PA, on November 12, 2011.

Disclosures

R.S.P. has received research funding from Abbvie Laboratories. C.C. has consulted for Merck on statistical methods for the analysis of electroencephalographic data and for OnX Life Technologies on statistical methods for the design of adaptive clinical trials on mitral valve implants.

References

1. United States Renal Data System: *USRDS 2011 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States*, Bethesda, MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2011
2. Ibels LS, Alfrey AC, Huffer WE, Craswell PW, Anderson JT, Weil R 3rd: Arterial calcification and pathology in uremic patients undergoing dialysis. *Am J Med* 66: 790–796, 1979
3. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, Salusky IB: Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 342: 1478–1483, 2000
4. Mizobuchi M, Towler D, Slatopolsky E: Vascular calcification: the killer of patients with chronic kidney disease. *J Am Soc Nephrol* 20: 1453–1464, 2009
5. Moe SM, Chen NX: Mechanisms of vascular calcification in chronic kidney disease. *J Am Soc Nephrol* 19: 213–216, 2008
6. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM: Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 87: E10–E17, 2000
7. Davies MR, Lund RJ, Mathew S, Hruska KA: Low turnover osteodystrophy and vascular calcification are amenable to skeletal anabolism in an animal model of chronic kidney disease and the metabolic syndrome. *J Am Soc Nephrol* 16: 917–928, 2005
8. Speer MY, McKee MD, Guldberg RE, Liaw L, Yang H-Y, Tung E, Karsenty G, Giachelli CM: Inactivation of the osteopontin gene enhances vascular calcification of matrix Gla protein-deficient mice: Evidence for osteopontin as an inducible inhibitor of vascular calcification in vivo. *J Exp Med* 196: 1047–1055, 2002
9. Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W: The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *J Clin Invest* 112: 357–366, 2003
10. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS: Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 12: 1260–1268, 1998
11. Jahnen-Dechent W, Heiss A, Schäfer C, Ketteler M: Fetuin-A regulation of calcified matrix metabolism. *Circ Res* 108: 1494–1509, 2011
12. Hruska KA, Mathew S, Saab G: Bone morphogenetic proteins in vascular calcification. *Circ Res* 97: 105–114, 2005

13. Giachelli CM, Steitz S: Osteopontin: A versatile regulator of inflammation and biomineralization. *Matrix Biol* 19: 615–622, 2000
14. Moe SM, Reslerova M, Ketteler M, O'neill K, Duan D, Koczman J, Westenfeld R, Jahnen-Dechent W, Chen NX: Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). *Kidney Int* 67: 2295–2304, 2005
15. Giachelli CM, Bae N, Almeida M, Denhardt DT, Alpers CE, Schwartz SM: Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. *J Clin Invest* 92: 1686–1696, 1993
16. Maréchal C, Schlieper G, Nguyen P, Krüger T, Coche E, Robert A, Floege J, Goffin E, Jadoul M, Devuyst O: Serum fetuin-A levels are associated with vascular calcifications and predict cardiovascular events in renal transplant recipients. *Clin J Am Soc Nephrol* 6: 974–985, 2011
17. Scialla JJ, Leonard MB, Townsend RR, Appel L, Wolf M, Budoff MJ, Chen J, Lustigova E, Gadegbeku CA, Glenn M, Hanish A, Raj D, Rosas SE, Seliger SL, Weir MR, Parekh RS; CRIC Study Group: Correlates of osteoprotegerin and association with aortic pulse wave velocity in patients with chronic kidney disease. *Clin J Am Soc Nephrol* 6: 2612–2619, 2011
18. Shroff RC, Shah V, Hiorns MP, Schoppet M, Hofbauer LC, Hawa G, Schurgers LJ, Singhal A, Merryweather I, Brogan P, Shanahan C, Deanfield J, Rees L: The circulating calcification inhibitors, fetuin-A and osteoprotegerin, but not matrix Gla protein, are associated with vascular stiffness and calcification in children on dialysis. *Nephrol Dial Transplant* 23: 3263–3271, 2008
19. Barreto DV, Barreto FC, Carvalho AB, Cuppari L, Cendoroglo M, Draibe SA, Moyses RMA, Neves KR, Jorgetti V, Blair A, Guiberteau R, Fernandes Canziani ME: Coronary calcification in hemodialysis patients: The contribution of traditional and uremia-related risk factors. *Kidney Int* 67: 1576–1582, 2005
20. Morena M, Dupuy A-M, Jaussent I, Vernhet H, Gahide G, Klouche K, Bargnoux A-S, Delcourt C, Canaud B, Cristol J-P: A cut-off value of plasma osteoprotegerin level may predict the presence of coronary artery calcifications in chronic kidney disease patients. *Nephrol Dial Transplant* 24: 3389–3397, 2009
21. Aryan M, Kepez A, Atalar E, Hazirolan T, Haznedaroglu İ, Akata D, Ozer N, Aksoyek S, Ovunc K, Ozmen F: Association of plasma osteopontin levels with coronary calcification evaluated by tomographic coronary calcium scoring. *J Bone Miner Metab* 27: 591–597, 2009
22. Ix JH, Chertow GM, Shlipak MG, Brandenburg VM, Ketteler M, Whooley MA: Association of fetuin-A with mitral annular calcification and aortic stenosis among persons with coronary heart disease: Data from the Heart and Soul Study. *Circulation* 115: 2533–2539, 2007
23. Miskulin DC, Martin AA, Brown R, Fink NE, Coresh J, Powe NR, Zager PG, Meyer KB, Levey AS; Medical Directors, Dialysis Clinic, Inc: Predicting 1 year mortality in an outpatient haemodialysis population: A comparison of comorbidity instruments. *Nephrol Dial Transplant* 19: 413–420, 2004
24. Liu Y, Coresh J, Eustace JA, Longenecker JC, Jaar B, Fink NE, Tracy RP, Powe NR, Klag MJ: Association between cholesterol level and mortality in dialysis patients: Role of inflammation and malnutrition. *JAMA* 291: 451–459, 2004
25. Royston P: Multiple imputation of missing values: update of ice. *Stata J* 5: 527–536, 2005
26. Newson R: Comparing the predictive power of survival models using Harrell's C or Somers' D. *Stata J* 10: 339–358, 2010
27. Morena M, Terrier N, Jaussent I, Leray-Moragues H, Chalabi L, Rivory J-P, Maurice F, Delcourt C, Cristol J-P, Canaud B, DupuyA-M: Plasma osteoprotegerin is associated with mortality in hemodialysis patients. *J Am Soc Nephrol* 17: 262–270, 2006
28. Reid P, Holen I: Pathophysiological roles of osteoprotegerin (OPG). *Eur J Cell Biol* 88: 1–17, 2009
29. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ: Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. *Cell* 89: 309–319, 1997
30. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ: Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 93: 165–176, 1998
31. Hanada S, Ando R, Naito S, Kobayashi N, Wakabayashi M, Hata T, Sasaki S: Assessment and significance of abdominal aortic calcification in chronic kidney disease. *Nephrol Dial Transplant* 25: 1888–1895, 2010
32. Olesen P, Ledet T, Rasmussen LM: Arterial osteoprotegerin: increased amounts in diabetes and modifiable synthesis from vascular smooth muscle cells by insulin and TNF-α. *Diabetologia* 48: 561–568, 2005
33. Boström KI, Jumabay M, Matveyenko A, Nicholas SB, Yao Y: Activation of vascular bone morphogenetic protein signaling in diabetes mellitus. *Circ Res* 108: 446–457, 2011
34. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C: Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int* 55: 648–658, 1999
35. Gutiérrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, Smith K, Lee H, Thadhani R, Jüppner H, Wolf M: Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med* 359: 584–592, 2008
36. Jean G, Terrat J-C, Vanel T, Hurot J-M, Lorriaux C, Mayor B, Chazot C: High levels of serum fibroblast growth factor (FGF)-23 are associated with increased mortality in long haemodialysis patients. *Nephrol Dial Transplant* 24: 2792–2796, 2009
37. Desjardins L, Liabeuf S, Renard C, Lenglet A, Lemke HD, Choukroun G, Drueke T, Massy Z: FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. *Osteoporos Int* 23: 2017–2025, 2012
38. Jean G, Bresson E, Lorriaux C, Mayor B, Hurot JM, Deleaval P, Chazot C: Increased levels of serum parathyroid hormone and fibroblast growth factor-23 are the main factors associated with the progression of vascular calcification in long-hour hemodialysis patients. *Nephron Clin Pract* 120: c132–c138, 2012
39. Nasrallah MM, El-Shehaby AR, Salem MM, Osman NA, El Sheikh E, Sharaf El Din UA: Fibroblast growth factor-23 (FGF-23) is independently correlated to aortic calcification in haemodialysis patients. *Nephrol Dial Transplant* 25: 2679–2685, 2010
40. Scialla JJ, Lau WL, Reilly MP, Isakova T, Yang HY, Crouthamel MH, Chavkin NW, Rahman M, Wahl P, Amaral AP, Hamano T, Master SR, Nessel L, Chai B, Xie D, Kallem RR, Chen J, Lash JP, Kusek JW, Budoff MJ, Giachelli CM, Wolf M; Chronic Renal Insufficiency Cohort Study Investigators: Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. *Kidney Int* 83: 1159–1168, 2013
41. Lim K, Lu TS, Molostvov G, Lee C, Lam FT, Zehnder D, Hsiao LL: Vascular Klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation* 125: 2243–2255, 2012
42. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, Gutiérrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, Feldman HI, St John Sutton M, Ojo A, Gadegbeku C, Di Marco GS, Reuter S, Kentrup D, Tiemann K, Brand M, Hill JA, Moe OW, Kuro-O M, Kusek JW, Keane MG, Wolf M: FGF23 induces left ventricular hypertrophy. *J Clin Invest* 121: 4393–4408, 2011
43. Honda H, Qureshi AR, Heimbürger O, Barany P, Wang K, Pecoits-Filho R, Stenvinkel P, Lindholm B: Serum albumin, C-reactive protein, interleukin 6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. *Am J Kidney Dis* 47: 139–148, 2006
44. Hermans MMH, Brandenburg V, Ketteler M, Kooman JP, van der Sande FM, Boeschoten EW, Leunissen KML, Krediet RT, Dekker FW; Netherlands cooperative study on the adequacy of Dialysis (NECOSAD): Association of serum fetuin-A levels with mortality in dialysis patients. *Kidney Int* 72: 202–207, 2007
45. Wang AY-M, Woo J, Lam CW-K, Wang M, Chan IH-S, Gao P, Lui S-F, Li PK-T, Sanderson JE: Associations of serum fetuin-A with malnutrition, inflammation, atherosclerosis and valvular

calcification syndrome and outcome in peritoneal dialysis patients. *Nephrol Dial Transplant* 20: 1676–1685, 2005
46. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Böhm R, Metzger T, Wanner C, Jahnen-Dechent W, Floege J: Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: A cross-sectional study. *Lancet* 361: 827–833, 2003
47. Stenvinkel P, Wang K, Qureshi AR, Axelsson J, Pecoits-Filho R, Gao P, Barany P, Lindholm B, Jogestrand T, Heimbürger O, Holmes C, Schalling M, Nordfors L: Low fetuin-A levels are associated with cardiovascular death: Impact of variations in the gene encoding fetuin. *Kidney Int* 67: 2383–2392, 2005
48. Ix JH, Shlipak MG, Sarnak MJ, Beck GJ, Greene T, Wang X, Kusek JW, Collins AJ, Levey AS, Menon V: Fetuin-A is not associated with mortality in chronic kidney disease. *Kidney Int* 72: 1394–1399, 2007
49. Jensen MK, Bartz TM, Mukamal KJ, Djoussé L, Kizer JR, Tracy RP, Zieman SJ, Rimm EB, Siscovick DS, Shlipak M, Ix JH: Fetuin-A, type 2 diabetes, and risk of cardiovascular disease in older adults: The cardiovascular health study. *Diabetes Care* 36: 1222–1228, 2013
50. Laughlin GA, Cummins KM, Wassel CL, Daniels LB, Ix JH: The association of fetuin-A with cardiovascular disease mortality in older community-dwelling adults: The Rancho Bernardo study. *J Am Coll Cardiol* 59: 1688–1696, 2012
51. Parker BD, Schurgers LJ, Brandenburg VM, Christenson RH, Vermeer C, Ketteler M, Shlipak MG, Whooley MA, Ix JH: The associations of fibroblast growth factor 23 and uncarboxylated matrix Gla protein with mortality in coronary artery disease: The Heart and Soul Study. *Ann Intern Med* 152: 640–648, 2010
52. Smith ER, Ford ML, Tomlinson LA, Rocks BF, Rajkumar C, Holt SG: Poor agreement between commercial ELISAs for plasma fetuin-A: An effect of protein glycosylation? *Clin Chim Acta* 411: 1367–1370, 2010
53. Hofbauer LC, Schoppet M: Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases. *JAMA* 292: 490–495, 2004
54. Scatena M, Liaw L, Giachelli CM: Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease. *Arterioscler Thromb Vasc Biol* 27: 2302–2309, 2007
55. Hamano T, Matsui I, Mikami S, Tomida K, Fujii N, Imai E, Rakugi H, Isaka Y: Fetuin-mineral complex reflects extraosseous calcification stress in CKD. *J Am Soc Nephrol* 21: 1998–2007, 2010

Received: May 20, 2013 **Accepted:** November 11, 2013

Published online ahead of print. Publication date available at www.cjasn.org.

This article contains supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.05450513/-/DCSupplemental.