Check for updates

Calcification Biomarkers, Subclinical Vascular Disease, and Mortality Among Multiethnic Dialysis Patients

Jessica Fitzpatrick[1], Esther D. Kim[2,3], Stephen M. Sozio[3,4], Bernard G. Jaar[2,3,4,5], Michelle M. Estrella[6,7], Jose M. Monroy-Trujillo[4] and Rulan S. Parekh[1,2,4,8]

[1]Child Health Evaluative Sciences, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada; [2]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA; [3]Welch Center for Prevention, Epidemiology, and Clinical Research, Baltimore, Maryland, USA; [4]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; [5]Nephrology Center of Maryland, Baltimore, Maryland, USA; [6]Kidney Health Research Collaborative, Department of Medicine, University of California, San Francisco, San Franscisco, California, USA; [7]Department of Medicine, San Francisco VA Medical Center, San Francisco, California, USA; and [8]Division of Nephrology, Department of Pediatrics and Medicine, The Hospital for Sick Children, University Health Network and University of Toronto, Ontario, Canada

Introduction: Vascular calcification and stiffness are associated with higher mortality and cardiovascular disease in hemodialysis patients, but the underlying mechanism is not well elucidated and previous studies have been contradictory. We sought to determine the association of circulating calcification biomarkers with calcification, stiffness, and mortality in a multiethnic incident dialysis population.

Methods: Among 391 incident hemodialysis participants enrolled in the Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease (PACE) study, we examined the cross-sectional associations of baseline fibroblast growth factor 23 (FGF23), desphospho-uncarboxylated matrix Gla protein (dp-ucMGP), fetuin-A, and osteoprotegerin (OPG) according to total coronary artery calcium score (CAC, using the Agatston calcification criteria) at baseline, vascular stiffness (pulse wave velocity [PWV]) over 4 study visits, and all-cause mortality.

Results: Patients' mean age was 55 years; 40% were female, 72% were African American, and 58% had diabetes. Higher OPG and FGF23 were associated with a 1.09-fold (per 5-pmol/l increase in OPG; 95% confidence interval [CI]: 1.01–1.17) and 1.12-fold (per increase of 100 log RU/ml in FGF23; 95% CI: 1.02–1.34) higher prevalence of CAC, independent of demographics, comorbidities, dialysis factors, and serum klotho levels. Higher OPG was associated with higher baseline PWV. Higher FGF23 was associated with lower PWV over follow-up. dp-ucMGP and fetuin-A were not associated with either CAC or vascular stiffness. After adjustment, circulating biomarkers were not associated with mortality risk.

Conclusion: Several circulating calcification biomarkers were only modestly associated with subclinical cardiovascular disease in an incident multiethnic hemodialysis population; none were associated with mortality. Understanding whether these associations persist in larger, diverse hemodialysis populations is warranted before planning trials.

P atients with chronic kidney disease (CKD), particularly those undergoing hemodialysis for end-stage renal disease (ESRD), are at elevated risk of cardiovascular morbidity and mortality. Worsening of vascular calcification and stiffness is associated with development of cardiovascular disease (CVD). The degree of vascular calcification and arterial stiffening are predictors of CVD mortality in both general and CKD populations.[1,2] Easily measured factors, such as circulating biomarkers, that report on vascular calcification may be useful to screen and target high-risk individuals.

As kidney function declines, phosphate is less efficiently removed from the serum and combines with calcium to form insoluble calcium phosphate, a component of vascular plaque. Furthermore, elevated serum phosphate levels stimulate osteoblastic differentiation of vascular cells, which leads to enhanced vascular plaque deposition and subsequent blood

Correspondence: Rulan S. Parekh, Child Health Evaluative Services, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada. E-mail: rulan.parekh@sickkids.ca

vessel stiffening.[3,4] Matrix Gla protein (MGP), fetuin A, osteoprotegerin (OPG), and fibroblast growth factor 23 (FGF23) are circulating factors that regulate these processes and have been investigated as biomarkers of vascular calcification and CVD risk. Posttranslationally phosphorylated and carboxylated MGP secreted by vascular smooth muscle cells inhibits calcium-phosphate precipitation in blood vessels and antagonizes proteins involved in bone formation.[5–8] The inactive dephosphorylated and uncarboxylated form of MGP (dp-ucMGP) can be readily detected in serum and has been associated with vascular calcification and CKD progression.[9] Fetuin-A influences vascular calcification by binding to calcium phosphate, which prevents its deposition in arterial plaques and inhibits vascular osteoblastic differentiation.[5,10,11] OPG regulates osteoclast precursor cell differentiation, including in blood vessel walls and bone, into osteoblasts.[5,12,13] FGF23 is involved in phosphate homeostasis, acts upon the kidneys to stimulate urinary phosphate excretion and inhibits 1,25-dihydroxyvitamin D production. It exerts its effect by binding to a complex of the FGF receptor and coreceptor, klotho.[5,14]

Previous clinical investigations of associations between levels of these biomarkers and vascular calcification or calcification-related outcomes in CKD have yielded inconsistent results.[5,9,15–18] These inconsistencies may arise from differences in patient populations as the majority of previous studies had small sample sizes and, importantly, comprised populations predominantly of European descent. Furthermore, extension of results from prevalent to incident hemodialysis is complicated by survival bias.

In this study we aimed to determine: (i) the association between dp-ucMGP, OPG, fetuin-A, and FGF23 and arterial stiffness and vascular calcification in a large multiethnic incident hemodialysis population; and (ii) the association of circulating biomarkers and increased mortality risk.

METHODS

Study Population
Concentrations of circulating biomarkers were measured in incident hemodialysis patients enrolled in the Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease study.[19] Five hundred sixty-eight participants were recruited from 27 dialysis units in Baltimore, Maryland, from November 2008 to August 2012. Inclusion criteria were: (i) ≥18 years of age and (ii) ability to speak English. Exclusion criteria were: (i) institutionalized persons; (ii) persons with a cancer diagnosis other than nonmelanoma skin cancer;

(iii) persons with a pacemaker or an automatic implantable cardioverter-defibrillator; and (iv) pregnant or nursing women. Participants who provided blood samples at the baseline clinic visit ($N = 391$) were eligible for the present study. Supplementary Figure S1 illustrates the participant flow for the current study. The study protocol was approved by the institutional review board at Johns Hopkins University, MedStar Health Systems, and the medical director of each dialysis unit. All participants provided written informed consent.

Data Collection and Measurements
At study enrollment, sociodemographic characteristics and medical history were collected through standardized questionnaires. Comorbidities were assessed by medical chart review, adjudicated, and classified using the Charlson Comorbidity Index. Systolic and diastolic blood pressures were obtained by averaging 3 consecutive seated-position measurements on a nondialysis day. Laboratory measures of serum albumin, serum creatinine, and single-pool Kt/V were collected as previously described, and the values reflected the 90-day average from dialysis initiation. Vitamin K and phosphate intakes were assessed by the standardized 24-hour dietary recall method with a dietician. High-sensitivity C-reactive protein (CRP) was measured by enzyme-linked immunoassay (ELISA) (coefficient of variation [CV] = 7%; BNII Nephelometer, Siemens Healthcare, Munich, Germany); and soluble klotho by ELISA (CV = 2.3%; Immuno-biological Laboratories, Takasaki, Japan) from fasting blood samples obtained at baseline.

Biomarker Assessment
Serum concentrations of dp-ucMGP, OPG, fetuin-A, and FGF23 were measured from fasting blood samples obtained at baseline on a nondialysis day. ELISA was used to measure dp-ucMGP (CV = 11%; VitaK BV, Maastricht, The Netherlands), OPG (CV = 9%; Alpaco Diagnostics, Salem, NH), fetuin-A (CV = 18%; Epitope Diagnostics, San Diego, CA), and C-terminal FGF23 (CV = 13%; Immutopics, San Clemente, CA) on samples stored at −80 °C.

Outcomes
Arterial stiffness was measured by carotid-femoral pulse wave velocity (PWV), which is considered a reference measure of central arterial stiffness. Measures were performed supine on the nonfistula arm using a PWV analysis system (Sphygmocor PVx, AtCor Medical, Sydney, Australia). PWV was measured at yearly clinic visits (4 visits total) and log-transformed for analysis. Baseline PWV measurements were obtained

for 293 participants. PWV measurements were available for 164 participants at year 1, 54 at year 2, and 16 at year 3. Coronary artery calcium (CAC) score was measured at baseline using computed tomography (Aquilon 32, Toshiba, Minato, Japan) and scored according to the Agatston calcification criteria.[20] Total CAC score was defined as the sum of left main, left anterior descending, left circumflex, and right coronary arteries. Prevalent calcification was defined as a CAC score >0. Baseline CAC scores were obtained for 305 participants.

Vital status was ascertained from the Standard Analysis Files of the United States Renal Data System. Participants were followed until December 31, 2017 ($n = 116$), death ($n = 179$), transplant ($n = 67$), transfer to peritoneal dialysis ($n = 18$), or loss to follow-up ($n = 11$).

Statistical Analysis

Participants' characteristics at study enrollment were summarized using mean and standard deviation, median and interquartile range, or frequency and proportion, as appropriate. Correlations among circulating biomarkers of calcification, phosphorus homeostasis, and inflammation and procalcification factors were estimated.

Associations of dp-ucMGP, OPG, fetuin-A, and FGF23 with baseline PWV were estimated separately using linear regression. dp-ucMGP and FGF23 were log-transformed before analysis. Potential confounders were identified *a priori* based on previous studies of calcification in ESRD with final variable selection conducted on the basis of *P* values ($P < 0.25$) from univariable analyses and changes in effect size. Candidate covariates included demographics (age, sex, race); traditional CVD risk factors (Charlson Comorbidity Index, body mass index, smoking status, history of hypercholesterolemia, systolic blood pressure, number of antihypertensive medications); and concentrations of serum albumin, CRP, corrected calcium, phosphate, parathyroid hormone [PTH], and dietary phosphate intake. The dp-ucMGP models were further adjusted for dietary vitamin K intake and FGF23 models for serum klotho concentration. Age, body mass index, blood pressure, corrected calcium, phosphate, PTH, dietary vitamin K, and phosphate intake were modeled as continuous variables and CRP was log-transformed before analysis. The association of dp-ucMGP, OPG, fetuin-A, and FGF23 with PWV during follow-up was estimated using linear mixed-effects regression with random intercepts to account for intraindividual correlation. An analogous approach was used to estimate the association between dp-ucMGP, OPG, fetuin-A, and FGF23 with

prevalence of CAC at baseline using Poisson regression with robust variance. Among participants with prevalent CAC at baseline, we estimated the association of dp-ucMGP, OPG, fetuin-A, and FGF23 with severity of calcification using log-linear regression. We separately examined nonlinear relationships between each biomarker and PWV, prevalence of CAC, and severity of CAC by including restricted cubic splines with knots at the 10th, 50th, and 90th quantiles in adjusted models.

In sensitivity analyses, we tested whether the associations between dp-ucMGP, OPG, fetuin-A, and FGF23 and PWV over follow-up were robust enough to include only baseline and year 1 visits.

In secondary analyses, the associations between dp-ucMGP, OPG, fetuin-A, and FGF23 and all-cause mortality were evaluated separately using Cox proportional hazards regression. These models were verified using scaled Schoenfeld residuals, and the linearity of continuous variables was assessed with Martingale residuals vs. fitted values plots.

To examine potential effect modifications in the associations of circulating biomarkers with PWV, prevalence of CAC, and mortality, we performed subgroup analyses by different categories of diabetes, race, and CRP, on the basis of *a priori* hypotheses. We tested for statistical interactions between each biomarker and the subgroups using likelihood ratio tests.

All missing covariate data were imputed using multiple imputation by the chained equations method with 20 imputations and 20 iterations.[21] Imputed variables included body mass index (1.2%), smoking status (0.3%), systolic and diastolic blood pressures (0.8%), serum phosphate (0.8%), corrected serum calcium (0.8%), calcium-phosphate product (0.8%), serum albumin (0.8%), PTH (0.5%), and vitamin K intake (22.0%). Statistical analyses were performed using R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Participants' Characteristics

Among 391 adults initiating hemodialysis, the mean age was 55 ± 13 years and the majority were male (60%) and African American (72%) (Table 1). The most common comorbidities were hypertension (100%), diabetes (58%), congestive heart failure (41%), and coronary artery disease (37%). Correlation analysis revealed that serum dp-ucMGP was significantly correlated with fetuin-A (−0.13), and FGF23 (0.12). All other pairwise correlations were not significant (Table 2).

Table 1. Baseline characteristics of PACE participants (2008–2012)

Variables	N	Mean (± SD), median (IQR), or frequency (%)
Demographics		
Total number	391	
Age, years		55.0 ± 13.2
Sex		
Male		234 (60)
Female		157 (40)
Race		
African American		283 (72)
Non–African American		108 (28)
Cardiovascular disease risk factors		
Body mass index, kg/m^2	386	29.6 ± 7.8
Hypercholesterolemia	391	269 (69)
Ever smoker	390	236 (61)
Diabetes mellitus	391	225 (58)
Coronary artery disease	391	143 (37)
Congestive heart failure	391	161 (41)
Charlson comorbidity index	391	5 (4–6)
Systolic blood pressure, mm Hg	388	137.6 ± 25.5
Diastolic blood pressure, mm Hg	388	74.7 ± 14.7
Dialysis laboratory measurements[a]		
Serum phosphate, mg/dl	388	4.97 ± 1.04
Corrected serum calcium, mg/dl	388	8.94 ± 0.49
Calcium-phosphate product, mg^2/dl^2	388	42.4 ± 8.76
Serum albumin, g/dl	388	3.54 ± 0.48
PTH, pg/ml	389	420 (272–611)
Biochemical measurements		
dp-ucMGP, ppm	364	1412 (957–2070)
Osteoprotegerin, pmol/l	383	10.9 ± 4.9
Fetuin-A, g/l	383	0.51 ± 0.17
FGF23, RU/ml	382	527 (241–1125)
Klotho, pg/ml	367	365 (273–500)
C-reactive protein, μg/ml	383	5.86 (2.30–15.1)
Baseline dietary intake		
Vitamin K, μg	305	44.4 (24.3–98.5)
Phosphate, mg	326	779.8 (546.0–1108.0)

dp-ucMGP, dephosphorylated-uncarboxylated matrix Gla protein; FGF23, fibroblast growth factor 23; IQR, interquartile range; PTH, parathyroid hormone; SD, standard deviation.
[a]Corresponds to 3-month average from start of dialysis.

Biomarkers of Calcification and PWV

Median PWV was 10.1 m/s (interquartile range: 8.0–12.6) and was higher among diabetic compared with nondiabetic hemodialysis participants (Supplementary Figure S1). Higher fetuin-A concentration was associated with lower PWV at baseline and over follow-up in univariable analyses, but these associations did not persist after adjustment for demographic characteristics (Table 3). Similarly, higher FGF23 levels were associated with lower baseline PWV in unadjusted models, but not in adjusted models. Higher levels of OPG were associated with increased baseline PWV independently of demographics; however, this association was attenuated after further adjustment for CVD risk factors and inflammatory markers. Furthermore, each 5-pmol/l increase in baseline OPG concentration was associated

with a 3.7% increase in PWV over 4 years of observation (95% CI: 0.5–7.0), independent of demographics, CVD risk factors, and inflammatory markers. Each increase in 100 log RU/ml for FGF23 was also associated with a 2.9% decrease in PWV over follow-up (95% CI: 0.1–5.7), independent of demographic characteristics, CVD risk factors, markers of inflammation, markers of bone mineral metabolism, and serum klotho concentration. Serum klotho and dp-ucMGP were not associated with PWV in baseline and longitudinal analyses. There was no evidence of an interaction between any of the biomarkers and history of diabetes or CRP levels ($P_{interaction} > 0.25$) and patterns of associations were similar when stratified by diabetes (Supplementary Table S1). When examined by subgroups of race, OPG concentration was independently associated with an increase in PWV over follow-up among African Americans but not in non–African Americans; however, the interaction between OPG and race was not statistically significant (Supplementary Table S2). There was no evidence of nonlinear associations between biomarkers and PWV (all $P_{nonlinearity} > 0.2$). Similar results were obtained when analyses were restricted to the first year of follow-up (Supplementary Table S3).

Biomarkers of Calcification and Prevalence of Calcification

Median CAC score was 42 (interquartile range: 0–463), and 64% of the participants with measured CAC scores had an abnormal score (CAC > 0) at baseline. Distribution of the CAC score was similar among those with and without diabetes (Supplementary Figure S1). Each 5-pmol/l higher OPG was associated with 1.11-fold greater prevalence of abnormal CAC score at baseline (95% CI: 1.03–1.19), independent of demographics (Table 3). OPG remained associated with baseline CAC score after further adjustment for CVD risk factors and inflammation markers (5-pmol/l increase; propensity ratio = 1.09; 95% CI: 1.01–1.17). Each 100 log RU/ml higher FGF23 was associated with a 1.12-fold increased prevalence of an abnormal CAC score (95% CI: 1.02–1.34), independent of demographics, cardiovascular risk factors, inflammation markers, bone mineral metabolism markers, and serum klotho. Serum klotho was not associated with an abnormal CAC score. In unadjusted analyses, each log part-per-million (ppm) higher dp-ucMGP was associated with a 1.12-fold greater prevalence of abnormal CAC score (95% CI: 1.00–1.25), but this association did not persist after adjusting for demographics. Fetuin-A was not associated with CAC score in the overall group, but, among those without diabetes, each 1-g/l higher fetuin-A concentration was associated with a 2.07-fold greater prevalence of calcification (95%

Table 2. Correlation among circulating biomarkers of calcification, phosphorus homeostasis, and inflammation and procalcification factors in adults initiating hemodialysis

	Correlations									
Variable	dp-ucMGP	OPG	Fetuin A	FGF23	Klotho	Corrected Ca	Phosphate	PTH	CRP	Albumin
dp-ucMGP	1.00									
OPG	0.01	1.00								
Fetuin-A	−0.13[a]	−0.06	1.00							
FGF23	0.12[a]	0.10	−0.03	1.00						
Klotho	0.00	0.06	0.02	0.04	1.00					
Corrected Ca	−0.00	0.04	0.04	0.12[a]	−0.00	1.00				
Phosphate	0.05	0.04	0.09	0.35[a]	−0.10	−0.20[a]	1.00			
PTH	−0.13[a]	−0.03	−0.02	0.23[a]	0.06	−0.27[a]	0.22[a]	1.00		
CRP	0.25[a]	0.03	−0.19[a]	0.05	0.05	−0.00	0.00	0.02	1.00	
Albumin	−0.10	−0.23[a]	0.20[a]	−0.12[a]	−0.09	−0.05	0.03	0.13[a]	−0.05	1.00

CRP, C-reactive protein; dp-ucMGP, desphospho-uncarboxylated matrix Gla protein; FGF23, fibroblast growth factor 23; HR, hazard ratio; OPG, osteoprotegerin; PTH, parathyroid hormone.
[a]$P < 0.05$ (statistically significant).

CI: 1.03–4.13). The associations were similar across diabetes history, race, and CRP levels (data not shown). There was no evidence of nonlinear associations between biomarkers and CAC (all $P_{\text{nonlinearity}} > 0.3$).

Among 195 participants with CAC > 0, none of the biomarkers were associated with severity of calcification (dp-ucMGP: 1 log ppm higher, 5.1% difference [95% CI: −42% to 33%]; OPG: 5 pmol/l higher, 2.9% difference [95% CI: −25% to 31%]; fetuin-A: 1 g/l higher, 12.8% difference [95% CI: −156% to 182%]; FGF23: 100 log RU/ml higher, 12% difference [95% CI: −185% to 420%]) after adjustment for demographics, CVD risk factors, and inflammation markers.

Biomarkers of Vascular Calcification and Mortality

During 1555.7 person-years, there were 179 deaths from all causes (incidence rate: 115.1 [95% CI: 98.8–133.2] per 1000 person-years). Median follow-up time was 3.5 (interquartile range: 1.7–6.2) years. In univariable analyses, higher levels of OPG at baseline were associated with increased risk of mortality (5 pmol/l higher; hazard ratio: 1.27 [95% CI: 1.13–1.42]). This association, however, did not persist after multivariable adjustment (5 pmol/l higher; hazard ratio = 1.08 [95% CI: 0.95–1.23]; Figure 1 and Table 4). dp-ucMGP, fetuin-A, FGF23, and serum klotho were not associated with mortality risk. There were no significant effect modifications by

Table 3. Associations of vascular and phosphate biomarkers with pulse wave velocity and coronary artery calcification in incident hemodialysis participants

	Pulse wave velocity (log m/s)			Coronary artery calcification	
Variables	N	Percent difference for baseline associations (95% CI)	Percent difference for associations over follow-up (95% CI)	N	Baseline associations PR (95% CI)
dp-ucMGP, log ppm					
Unadjusted	272	0.50 (−3.9 to 5.9)	−0.40 (−4.2–3.4)	281	1.12 (1.00–1.25)[a]
Model 1[b]	272	0.20 (−4.2 to 4.7)	−0.60 (−4.2–3.1)	281	1.03 (0.92–1.15)
Model 2[c]	272	0.30 (−3.9 to 4.5)	−1.3 (−5.2 to 2.5)	281	1.02 (0.92–1.14)
OPG, 5 pmol/l					
Unadjusted	287	10.0 (6.5–14.0)[a]	9.0 (5.5–12.0)[a]	298	1.22 (1.13–1.31)[a]
Model 1[b]	287	6.0 (2.0–9.5)[a]	5.0 (1.5–8.0)[a]	298	1.11 (1.03–1.19)[a]
Model 2[c]	287	3.0 (−0.50 to 7.0)	3.7 (0.50–7.0)[a]	298	1.09 (1.01–1.17)[a]
Fetuin-A, g/l					
Unadjusted	287	−20.7 (−40.7 to −8.0)[a]	−19.6 (−37.0 to −1.9)[a]	298	0.61 (0.34–1.08)
Model 1[b]	287	−6.6 (−25.6 to 12.4)	−6.5 (−22.6 to 9.6)	298	1.04 (0.62–1.73)
Model 2[c]	287	0.10 (−17.8 to 18.0)	−4.5 (−20.2 to 11.2)	298	1.25 (0.74–2.11)
FGF23, 100 log RU/ml					
Unadjusted	286	−4.9 (−8.7 to −1.0)[a]	−3.9 (−7.1 to −0.60)[a]	297	1.08 (0.98–1.18)
Model 1[b]	286	−2.5 (−6.1 to 1.2)	−2.1 (−5.0 to 0.80)	297	1.14 (1.05–1.25)[a]
Model 2[c]	286	−2.8 (−6.3 to 0.80)	−2.9 (−5.7 to −0.10)[a]	297	1.12 (1.02–1.34)[a]

dp-ucMGP, desphospho-uncarboxylated matrix Gla protein; FGF23, fibroblast growth factor 23; HR, hazard ratio; OPG, osteoprotegerin; PR, prevalence ratio.
[a]$P < 0.05$ (statistically significant).
[b]Model 1 is adjusted for model including age, sex, and race.
[c]Model 2 is adjusted for all variables in Model 1 plus the Charlson comorbidity index, albumin and C-reactive protein, body mass index, and systolic blood pressure; for dp-ucMGP, Model 2 is also adjusted for dietary vitamin K intake; for FGF23, Model 2 is also adjusted for serum soluble klotho and serum phosphate, and serum calcium corrected for albumin.
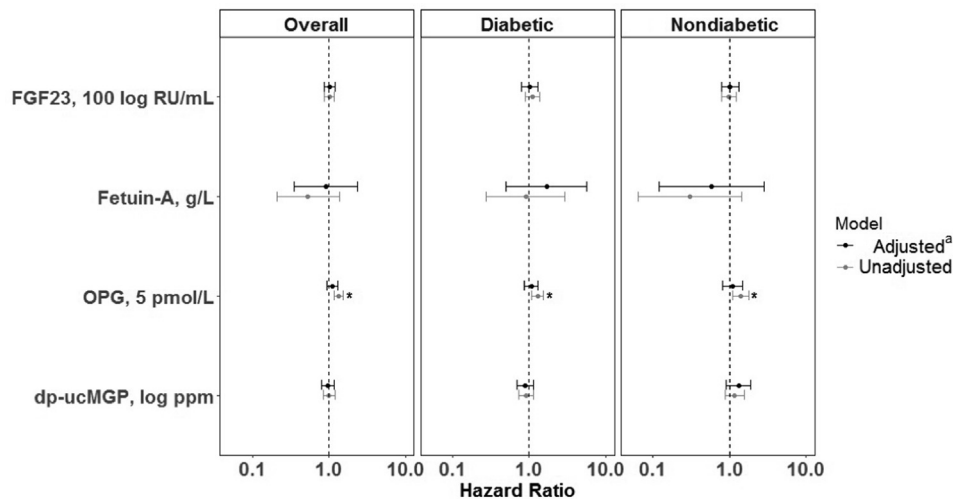
Figure 1. Associations of biomarkers with all-cause mortality among participants in the Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease (PACE) study. [a]Model adjusted for biomarkers, age, sex, race, comorbidity index, body mass index, albumin, and C-reactive protein; for desphospho-uncarboxylated matrix Gla protein (dp-ucMGP) model, also adjusted for dietary vitamin K intake; for fibroblast growth factor 23 (FGF23) model, also adjusted for serum klotho and phosphorus concentration. OPG, osteoprotegerin.

diabetes, race, or CRP levels. There was no evidence of nonlinear associations between biomarkers and mortality (all $P_{\text{nonlinearity}} > 0.3$).

DISCUSSION

In adults initiating hemodialysis, higher levels of OPG and FGF23 at baseline were associated with a modestly higher prevalence of abnormal CAC score. Higher levels of OPG at baseline were also associated with a 4% increase in arterial stiffness over time. Despite the modest association with subclinical CVD, there was no association between mortality and any of the circulating calcification biomarkers in this incident multiethnic hemodialysis population.

In contrast to many previous studies investigating the association of circulating biomarkers with calcification and mortality, our study population is predominantly African American. Data from the Multi-Ethnic Study of Atherosclerosis and other studies demonstrated that African Americans are at high risk of CVD events,[22,23] despite a lower prevalence of CAC[24] and thoracic aorta calcification.[25] This disparity highlights the need to investigate novel predictors of subclinical CVD in African Americans on hemodialysis.

OPG secreted by osteoblasts regulates differentiation of osteoclasts and promotes bone mineralization,[5,12,13] whereas OPG secreted from blood vessels prevents vascular mineralization. Elevated OPG levels in hemodialysis patients likely result from a compensatory mechanism of the endothelium to counteract vascular calcium-phosphate product deposition, or increased differentiation of vascular smooth muscle cells similar to osteoblasts.[5] Consistent with our results, previous studies have reported associations between elevated

levels of OPG and CAC score in ESRD populations. In cohorts of prevalent hemodialysis patients from Japan and Brazil, OPG was associated with increased calcification and arterial stiffness.[26,27] In contrast, a study of 87 prevalent hemodialysis patients from Romania showed that elevated OPG was not associated with vascular calcification.[28] Our study confirms associations of OPG with increased vascular calcification and stiffness in a larger cohort of American incident hemodialysis patients. In contrast to our study, previous research has shown consistent associations of OPG with increased mortality risk in prevalent[29–31] and incident[32] ESRD patients. The Choices for Healthy Outcomes in Caring for ESRD (CHOICE) study[32] showed that the highest OPG tertile was independently

Table 4. Associations of vascular and phosphate biomarkers all-cause mortality in incident hemodialysis participants

Variables	N	HR (95% CI)
dp-ucMGP, log ppm	364	
Unadjusted		1.00 (0.84–1.20)
Model 1[a]		0.98 (0.80–1.19)
OPG, 5 pmol/l	383	
Unadjusted		1.27 (1.13–1.42)[b]
Model 2[c]		1.08 (0.96–1.23)
Fetuin-A, g/l	383	
Unadjusted		0.54 (0.21–1.39)
Model 2[c]		0.92 (0.35–2.38)
FGF23, 100 log RU/ml	382	
Unadjusted		1.02 (0.88–1.85)
Model 2[c]		1.02 (0.86–1.20)

dp-ucMGP, desphospho-uncarboxylated matrix Gla protein; FGF23, fibroblast growth factor 23; HR, hazard ratio; OPG, osteoprotegerin.
[a]Model 1 is adjusted for model including age, sex, race, body mass index, Charlson Comorbidity Index, albumin, C-reactive protein, and dietary vitamin K intake.
[b]$P < 0.05$.
[c]Model 2 is adjusted for model including age, sex, race, body mass index, Charlson Comorbidity Index, albumin, and C-reactive protein.

associated with decreased survival. The differences between those earlier study results and ours may arise from the differences in patient populations; for example, 60% of the CHOICE study participants were Caucasian, and 72% of participants in the Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease study were African American. Moreover, the process and delivery of care have changed since the CHOICE study enrolled participants in the 1990s.

FGF23 plays a major role as a regulator of phosphate homeostasis by increasing urinary phosphate excretion upon interaction with the membrane-bound klotho-FGF23 receptor protein complex.[5,14] ESRD patients exhibit decreased levels of klotho and, consequently, FGF23 remains in the circulation at elevated levels, which may reflect levels of vascular calcification.[33] In agreement with our observed association between higher baseline levels of FGF23 and prevalent CAC, studies from China and Taiwan showed that FGF23 was associated with increased aortic calcification.[34] In the Chronic Renal Insufficiency Cohort (CRIC) study of 1501 patients with mild to moderate CKD, however, FGF23 was not associated with CAC score.[35] FGF23 was associated with increased mortality risk in the CRIC study, possibly mediated through left ventricular failure.[36] FGF23 was also associated with increased odds of mortality in 10,044 incident hemodialysis patients from the Accelerated Mortality of Renal Replacement (ArMORR) cohort,[37] but not in other studies.[38,39] We note that, with the exception of the ArMORR study, C-terminal FGF23 was measured in those studies and ours; the ArMORR study measured intact FGF23. We did not observe any association between FGF23 and mortality, which could suggest major ethnic differences in FGF23 and mineral metabolism that remain in question.[37] The increase in CAC with increasing FGF23 was not reflected in a corresponding increase in PWV; in fact, FGF23 was only modestly associated with a small decrease in PWV. It remains unclear why the increase in FGF23, which is associated with an increase in the single-timepoint assessment of CAC, would lead to a decrease in PWV at baseline and over time, but we are unable to rule out the possibility that this observation results from residual confounding.

MGP directly inhibits vascular calcification by solubilizing calcium-phosphate product, preventing its crystallization.[5–8] Activation of MGP requires a vitamin K–dependent posttranslational modification, and ongoing clinical trials in Europe and Canada are investigating the influence of vitamin K supplementation on the progression of CAC in hemodialysis.[40,41] Elevated levels of dp-ucMGP are indicative of depressed levels of active MGP, which cannot currently be assayed.[9] Although levels of dp-ucMGP have been associated with increased risk of calcification in prevalent dialysis[15,16] and CKD patients,[9] we observed no association of dp-ucMGP with CAC score or arterial stiffness independently of dietary vitamin K intake in incident hemodialysis patients. Previous studies in CKD patients indicated either no association[9] or a tenuous association[17] between dp-ucMGP and mortality and we extend these findings to incident hemodialysis patients. The majority of studies with positive findings were conducted in individuals of European descent, again suggesting possible differences in mechanisms leading to CVD mortality.

Similarly to MGP, fetuin-A binds to and solubilizes calcium and prevents its precipitation,[5,10,11] but current evidence concerning the association of fetuin-A levels with vascular calcification remains inconclusive. In 2 small studies, fetuin-A was associated with decreased vascular calcification.[42,43] In contrast, higher fetuin-A levels were associated with increased risk of abnormal CAC score in 72 prevalent hemodialysis patients in Turkey,[44] and not associated with vascular calcification in another study of 93 prevalent hemodialysis patients.[45] Although our unadjusted models showed fetuin-A to be associated with a 20% decrease in PWV, consistent with the hypothesis that increased fetuin-A results in solubilization of calcium-phosphate, a decrease in calcification, and a decrease in PWV, this association did not persist after adjustment. Moreover, no significant association of fetuin-A with CAC score was found in either our adjusted or unadjusted models.

The prospective nature of the cohort and inclusion of adults of all ages initiating hemodialysis represent key strengths of our study. Additional strengths include direct measures of CAC and arterial stiffness, a well-characterized cohort, adjudication of comorbidities, and a standardized protocol for cardiac evaluations. The cohort included a large proportion of African Americans from urban areas, who are often underrepresented in clinical studies. Limitations include the cross-sectional analyses between circulating biomarkers and CAC score and possible residual confounding. There is also a risk of selection bias due to the large proportion of participants lost to follow-up between baseline and year 1 (55%). Additionally, differences between patients with and without complete biomarker and outcome data are summarized in Supplementary Table S4. Finally, although we had sufficient statistical power (>70%) to detect associations between dp-ucMGP, OPG, or FGF23 and mortality, we may not have had adequate power to detect associations between fetuin-A and mortality.

Despite the high prevalence of vascular calcification in this cohort, we demonstrated that circulating biomarkers of calcification, such as OPG and FGF23, were

only modestly associated with intermediate measures of cardiovascular disease, specifically coronary calcification and vascular stiffness. No vascular calcification markers studied were significantly associated with mortality in an incident, multiethnic population. These markers may not be indicative of future risk in dialysis due to the advanced CVD incurred by prolonged CKD. The large proportion of African Americans in our study highlights the importance of considering differences in study groups when planning trials and determining factors to screen and target this high-risk dialysis population.

DISCLOSURE

All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)
Figure S1. Participant flowchart.
Figure S2. Distribution of baseline pulse wave velocity (A) and calcification (B) (no calcification: CAC = 0; calcification: CAC > 0) among diabetic and nondiabetic Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease study participants.
Table S1. Associations of vascular and phosphate biomarkers with pulse wave velocity and coronary artery calcification among diabetic and nondiabetic incident hemodialysis participants.
Table S2. Associations of desphospho-uncarboxylated matrix Gla protein with pulse wave velocity and coronary artery calcification among African American and non–African American incident hemodialysis participants.
Table S3. Associations of vascular and phosphate biomarkers with pulse wave velocity in incident hemodialysis participants with follow-up from baseline to year 1.
Table S4. Comparison of characteristics of participants with and without complete biomarker and outcome measure.

REFERENCES

1. Toussaint ND, Kerr PG. Vascular calcification and arterial stiffness in chronic kidney disease: implications and management. *Nephrology (Carlton)*. 2007;12:500–509.

2. Mackey RH, Venkitachalam L, Sutton-Tyrrell K. Calcifications, arterial stiffness and atherosclerosis. *Adv Cardiol*. 2007;44:234–244.

3. Moe SM, Chen NX. Mechanisms of vascular calcification in chronic kidney disease. *J Am Soc Nephrol*. 2008;19:213–216.

4. Cozzolino M, Brancaccio D, Gallieni M, Slatopolsky E. Pathogenesis of vascular calcification in chronic kidney disease. *Kidney Int*. 2005;68:429–436.

5. Liabeuf S, Okazaki H, Desjardins L, et al. Vascular calcification in chronic kidney disease: are biomarkers useful for probing the pathobiology and the health risks of this process in the clinical scenario? *Nephrol Dial Transplant*. 2014;29:1275–1284.

6. Luo G, Ducy P, McKee MD, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature*. 1997;386:78–81.

7. Lomashvili KA, Wang X, Wallin R, O'Neill WC. Matrix Gla protein metabolism in vascular smooth muscle and role in uremic vascular calcification. *J Biol Chem*. 2011;286:28715–28722.

8. Zebboudj AF, Imura M, Bostrom K. Matrix GLA protein, a regulatory protein for bone morphogenetic protein-2. *J Biol Chem*. 2002;277:4388–4394.

9. Schurgers LJ, Barreto DV, Barreto FC, et al. The circulating inactive form of matrix Gla protein is a surrogate marker for vascular calcification in chronic kidney disease: a preliminary report. *Clin J Am Soc Nephrol*. 2010;5:568–575.

10. Reynolds JL, Skepper JN, McNair R, et al. Multifunctional roles for serum protein fetuin-a in inhibition of human vascular smooth muscle cell calcification. *J Am Soc Nephrol*. 2005;16:2920–2930.

11. Schafer C, Heiss A, Schwarz A, et al. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *J Clin Invest*. 2003;112:357–366.

12. Simonet WS, Lacey DL, Dunstan CR, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*. 1997;89:309–319.

13. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *J Bone Miner Res*. 2000;15:2–12.

14. Zisman AL, Wolf M. Recent advances in the rapidly evolving field of fibroblast growth factor 23 in chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2010;19:335–342.

15. Delanaye P, Krzesinski JM, Warling X, et al. Dephosphory-lated-uncarboxylated Matrix Gla protein concentration is predictive of vitamin K status and is correlated with vascular calcification in a cohort of hemodialysis patients. *BMC Nephrol*. 2014;15:145.

16. Cranenburg EC, Brandenburg VM, Vermeer C, et al. Uncar-boxylated matrix Gla protein (ucMGP) is associated with coronary artery calcification in haemodialysis patients. *Thromb Haemost*. 2009;101:359–366.

17. Schlieper G, Westenfeld R, Kruger T, et al. Circulating non-phosphorylated carboxylated matrix gla protein predicts survival in ESRD. *J Am Soc Nephrol*. 2011;22:387–395.

18. Pichler G, Haller MC, Kainz A, Wolf M, Redon J, Oberbauer R. Prognostic value of bone- and vascular-derived molecular biomarkers in hemodialysis and renal transplant patients: a systematic review and meta-analysis. *Nephrol Dial Transplant*. 2017;32:1566–1578.

19. Parekh RS, Meoni LA, Jaar BG, et al. Rationale and design for the Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease (PACE) study. *BMC Nephrol*. 2015;16:63.

20. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15:827–832.

21. White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. *Stat Med*. 2011;30:377–399.

22. Budoff MJ, Young R, Burke G, et al. Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events: the multi-ethnic study of athero-sclerosis (MESA). *Eur Heart J*. 2018;39:2401–2408.

23. Doherty TM, Tang W, Detrano RC. Racial differences in the significance of coronary calcium in asymptomatic black and white subjects with coronary risk factors. *J Am Coll Cardiol*. 1999;34:787–794.

24. Bild DE, Detrano R, Peterson D, et al. Ethnic differences in coronary calcification: the Multi-Ethnic Study of Atheroscle-rosis (MESA). *Circulation*. 2005;111:1313–1320.

25. Youssef G, Guo M, McClelland RL, et al. Risk factors for the development and progression of thoracic aorta calcification: the multi-ethnic study of atherosclerosis. *Acad Radiol*. 2015;22:1536–1545.

26. Nitta K, Akiba T, Uchida K, et al. Serum osteoprotegerin levels and the extent of vascular calcification in haemodialysis pa-tients. *Nephrol Dial Transplant*. 2004;19:1886–1889.

27. Barreto DV, Barreto FC, Carvalho AB, et al. Coronary calci-fication in hemodialysis patients: the contribution of traditional and uremia-related risk factors. *Kidney Int*. 2005;67:1576–1582.

28. Moldovan D, Kacso IM, Rusu C, et al. Role of osteoprotegerin in vascular disorders of the end-stage renal disease patients. *Biomarkers*. 2015;20:116–122.

29. Winther S, Christensen JH, Flyvbjerg A, et al. Osteoprote-gerin and mortality in hemodialysis patients with cardiovas-cular disease. *Clin Nephrol*. 2013;80:161–167.

30. Morena M, Terrier N, Jaussent I, et al. Plasma osteoprote-gerin is associated with mortality in hemodialysis patients. *J Am Soc Nephrol*. 2006;17:262–270.

31. Speer G, Fekete BC, El Hadj Othmane T, et al. Serum osteo-protegerin level, carotid-femoral pulse wave velocity and cardiovascular survival in haemodialysis patients. *Nephrol Dial Transplant*. 2008;23:3256–3262.

32. Scialla JJ, Kao WH, Crainiceanu C, et al. Biomarkers of vascular calcification and mortality in patients with ESRD. *Clin J Am Soc Nephrol*. 2014;9:745–755.

33. Stubbs J, Liu S, Quarles LD. Role of fibroblast growth factor 23 in phosphate homeostasis and pathogenesis of disordered mineral metabolism in chronic kidney disease. *Semin Dial*. 2007;20:302–308.

34. Chen Z, Chen X, Xie J, et al. Fibroblast growth factor 23 is a predictor of aortic artery calcification in maintenance hemo-dialysis patients. *Ren Fail*. 2013;35:660–666.

35. Scialla JJ, Lau WL, Reilly MP, et al. Fibroblast growth factor 23 is not associated with and does not induce arterial calci-fication. *Kidney Int*. 2013;83:1159–1168.

36. Munoz Mendoza J, Isakova T, Cai X, et al. Inflammation and elevated levels of fibroblast growth factor 23 are independent risk factors for death in chronic kidney disease. *Kidney Int*. 2017;91:711–719.

37. Gutierrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med*. 2008;359:584–592.

38. Olauson H, Qureshi AR, Miyamoto T, et al. Relation between serum fibroblast growth factor-23 level and mortality in incident dialysis patients: are gender and cardiovascular disease confounding the relationship? *Nephrol Dial Trans-plant*. 2010;25:3033–3038.

39. Kendrick J, Cheung AK, Kaufman JS, et al. FGF-23 associates with death, cardiovascular events, and initiation of chronic dialysis. *J Am Soc Nephrol*. 2011;22:1913–1922.

40. Krueger T, Schlieper G, Schurgers L, et al. Vitamin K1 to slow vascular calcification in haemodialysis patients (VitaVasK trial): a rationale and study protocol. *Nephrol Dial Transplant*. 2014;29:1633–1638.

41. Holden RM, Booth SL, Day AG, et al. Inhibiting the pro-gression of arterial calcification with vitamin K in Hemo-Dialysis patients (iPACK-HD) trial: rationale and study design for a randomized trial of vitamin K in patients with end stage kidney disease. *Can J Kidney Health Dis*. 2015;2:17.

42. El-Shehaby AM, Zakaria A, El-Khatib M, Mostafa N. Associa-tion of fetuin-A and cardiac calcification and inflammation levels in hemodialysis patients. *Scand J Clin Lab Invest*. 2010;70:575–782.

43. Coen G, Pierantozzi A, Spizzichino D, et al. Risk factors of one year increment of coronary calcifications and survival in he-modialysis patients. *BMC Nephrol*. 2010;11:10.

44. Kirkpantur A, Altun B, Hazirolan T, et al. Association among serum fetuin-A level, coronary artery calcification, and bone mineral densitometry in maintenance hemodialysis patients. *Artif Organs*. 2009;33:844–854.

45. Ulutas O, Taskapan MC, Dogan A, Baysal T, Taskapan H. Vascular calcification is not related to serum fetuin-A and osteopontin levels in hemodialysis patients. *Int Urol Nephrol*. 2018;50:137–142.