

Association of Serum Alkaline Phosphatase with Coronary Artery Calcification in Maintenance Hemodialysis Patients

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Background and objectives: Recent *in vitro* studies have shown a link between alkaline phosphatase and vascular calcification in patients with chronic kidney disease (CKD). High serum levels of alkaline phosphatase are associated with increased death risk in epidemiologic studies of maintenance hemodialysis (MHD) patients. We hypothesized that coronary artery calcification is independently associated with increased serum alkaline phosphatase levels in MHD patients.

Design, setting, participants, & measurements: We examined the association of coronary artery calcification score (CACS) and alkaline phosphatase in 137 randomly selected MHD patients for whom markers of malnutrition, inflammation, and bone and mineral disorders were also measured.

Results: Serum alkaline phosphatase was the only measure with significant and robust association with CACS ($P < 0.003$), whereas either other biochemical markers had no association with CACS or their association was eliminated after controlling for case-mix variables. Serum alkaline phosphatase >120 IU/L was a robust predictor of higher CACS and was particularly associated with the likelihood of CACS >400 (multivariate odds ratio 5.0 95% confidence interval 1.6 to 16.3; $P = 0.007$). Serum alkaline phosphatase of approximately 85 IU/L seemed to be associated with the lowest likelihood of severe coronary artery calcification, but in the lowest tertile of alkaline phosphatase, the CACS predictability was not statistically significant.

Conclusions: An association between serum alkaline phosphatase level and CACS exists in MHD patients. Given the high burden of vascular calcification in patients with CKD, examining potential therapeutic interventions to modulate the alkaline phosphatase pathway may be warranted.

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Vascular calcification is common in individuals with chronic kidney disease (CKD) and is a significant correlate of the high cardiovascular death risk (1–3). Both *intimal* and *medial* calcification are observed frequently in patients with CKD (4–7). Several mechanisms have been implicated for the high prevalence of vascular calcification in CKD, including the high burden of conventional cardiovascular risks such as diabetes, hypertension, and dyslipidemia; bone and mineral disorders such as calcium load and secondary hyperparathyroidism (SHPT); chronic inflammation such as high level of proinflammatory cytokines; and deficiency of anticalcemic factors such as fetuin (8,9). Lomashvili *et al.* (5) reported that vascular damage can induce expression of tissue-nonspecific alkaline phosphatase (AlkPhos), which *per se* hydrolyzes and inactivates inorganic pyrophosphates, a process

that can enhance vascular calcification. We recently showed that higher serum AlkPhos levels in maintenance hemodialysis (MHD) patients were independently associated with increased all-cause and cardiovascular mortality in approximately 74,000 MHD patients, even after adjustment for surrogates of nutrition, inflammation, minerals, serum parathyroid hormone (PTH), and liver enzymes (10). Nevertheless, the pathophysiologic link between increased AlkPhos level and mortality in MHD patients is not clear. Given the recent *in vitro* findings about the link between AlkPhos and vascular calcification *via* the pyrophosphate pathway, we hypothesized that the increased cardiovascular and all-cause death risk observed in the setting of high serum AlkPhos level may be related to vascular calcification in MHD patients (6,11). To test this hypothesis, we examined the association of serum AlkPhos and coronary artery calcification in a cohort of MHD patients for whom other risk factors of cardiovascular disease including inflammatory markers were also examined.

Materials and Methods

Patient Population

We studied a randomly selected group of MHD patients who participated in the substudy of the Nutritional and Inflammatory Evaluation

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in Dialysis (NIED) Study (see the NIED Study website at <http://www.NIEDStudy.org> for more details, as well as previous publications [12–15]). The original patient cohort was derived from a pool of more than 3000 MHD outpatients during 5 yr in eight DaVita long-term dialysis facilities in the South Bay Los Angeles area. Inclusion criteria were outpatients who had been undergoing MHD for at least 8 wk, were ≥ 18 yr of age, and signed the institutional review board approved consent form. Patients with an anticipated life expectancy of < 6 mo (e.g., because of a metastatic malignancy or advanced HIV/AIDS disease) were excluded. From October 1, 2001, through December 31, 2006, 893 MHD patients signed the informed consent form and underwent the periodic evaluations of the NIED Study, and 176 of these individuals were randomly invited to undergo additional tests at the General Clinical Research Center at Harbor-UCLA as parts of the NIED Substudy (13). Out of the substudy, 153 patients had both coronary artery calcification assessment by electron beam computed tomography (EBCT) and AlkPhos measured.

The medical chart of each MHD patient was thoroughly reviewed by a collaborating physician, and data pertaining to the underlying kidney disease, cardiovascular history, and other comorbid conditions were extracted. A modified version of the Charlson comorbidity index (without the age and kidney disease components) was used to assess the severity of comorbidities (16,17).

EBCT Procedure and Interpretation

All patients underwent EBCT using an Imatron C-150XL ultrafast CT scanner (GE-Imatron, South San Francisco, CA) at Los Angeles Biomedical Research Institute at Harbor-UCLA, St. John's Cardiovascular Center. Tomographic imaging was electrocardiographically gated, and imaging acquisition occurred at a predetermined time in diastole (between 50 and 80% of the R-to-R cycle depending on heart rate). The coronary arteries were visualized without contrast medium, and 30 to 40 consecutive images were obtained at 3-mm intervals, from the bronchial carina caudally to include the entire coronary tree. A CT threshold of 3 pixels and 130 HU was used for identification of the calcified lesions. Specifically, a calcified lesion was defined as a minimum of three contiguous pixels ($0.56 \times 0.56 \times 3 = 1 \text{ mm}^3$ voxel) with a minimum attenuation of 130 HU. The number of calcified lesions was then totaled for each coronary artery. The total coronary artery calcification score was determined by summing individual lesion scores from each of anatomic sites (left main, left anterior descending, circumflex, and right coronary arteries) as described elsewhere (18).

Anthropometric and Dietary Measures

Body weight assessment and anthropometric measurements were performed while patients underwent a hemodialysis treatment or within 5 to 20 min after termination of the treatment. Biceps skinfold and triceps skinfold thicknesses were measured with a conventional skinfold caliper using standard techniques as described previously (19). Three-day diet recall with a subsequent interview was performed to estimate the total daily protein and calorie intake (20).

Near-Infrared Interactance

To estimate the percentage of body fat and fat-free body mass, we measured near infrared (NIR) interactance at the same time as the anthropometric measurements (21). A commercial NIR interactance sensor with a coefficient of variation of 0.5% for total body fat measurement (portable Futrex 6100, Gaithersburg, MD; <http://www.futrex.com>) was used. NIR measurements were performed by placing, for several seconds on the upper aspect of the arm without a vascular access, a Futrex sensor and entering the required data (date of birth,

gender, weight, and height) of each patient. NIR measurements of body fat seem to correlate significantly with other nutritional measures in MHD patients (22).

Laboratory Tests

Predialysis blood samples and postdialysis serum urea nitrogen were obtained on a midweek day that coincided chronologically with the drawing of quarterly blood tests in the DaVita facilities. The single-pool Kt/V was used to represent the weekly dialysis dosage. All routine laboratory measurements were performed by DaVita Laboratories (Deland, FL) using automated methods. Roche modular instrumentation method (23) (Roche Diagnostics Corp., Indianapolis, IN) was used for quantitative determinations of AlkPhos in that p-nitrophenyl phosphate is converted to p-nitrophenol plus phosphate, where p-nitrophenol released is proportional to the AlkPhos activity and is measured photometrically. Measured imprecision studies using DaVita patient samples recovered a coefficient of variation of $< 2.0\%$ and an extended reportable range of 1.0 to 4400.0 IU/L (Dr. J. Steinmetz, DaVita Laboratories, personal communication, April 2007).

Serum high-sensitivity C-reactive protein (CRP) was measured by a turbidometric immunoassay in which a serum sample is mixed with latex beads coated with anti-human CRP antibodies forming an insoluble aggregate (WPCI, Osaka, Japan; normal range $< 3.0 \text{ mg/L}$) (24,25). IL-6 and TNF- α were measured with immunoassay kits based on a solid-phase sandwich ELISA using recombinant human IL-6 and TNF- α (R&D Systems, Minneapolis, MN; normal range: IL-6 $< 9.9 \text{ pg/ml}$, TNF- α $< 4.7 \text{ pg/ml}$) (26,27). CRP, TNF- α , IL-1, and IL-6 were measured in the General Clinical Research Center Laboratories of Harbor-UCLA Medical Center. Serum transthyretin (prealbumin) was measured using immunoprecipitin analysis (15). Plasma total homocysteine concentrations were determined by HPLC in the Harbor-UCLA Clinical Laboratories.

Statistical Analysis

χ^2 test and linear regression analysis were used to examine the differences of proportion and trends of quantitative variables across tertiles of coronary artery calcification scores (CACs) after excluding those with no evidence of coronary artery calcification. Pearson correlation coefficient was used to examine the crude and adjusted linear correlation between Logarithm of CACS and AlkPhos as well as other relevant variables. Multivariate logistic regression models were fitted to construct odds ratio (OR) of CACS ≥ 400 in the first and third tertiles of serum AlkPhos before and after controlling for confounding covariates using middle tertile as the reference. Restricted cubic spline graphs were used as exploratory data analyses to illustrate systematic relations between serum AlkPhos and the likelihood of CACS ≥ 400 . This method also served to examine the nonlinear associations of continuous serum AlkPhos as an alternative to inappropriate linearity assumptions (28). Fiducial limits are given as means \pm SD; OR values include 95% confidence interval (CI) levels. $P < 0.05$ or a 95% CI that did not span 1.0 was considered to be statistically significant. $P = 0.05$ to 0.10 was considered to indicate a potentially significant trend to mitigate the chance of type II error (i.e., accepting the null hypothesis when it should be rejected). Descriptive and multivariate statistics were carried out with the statistical software Stata 10.0 (Stata Corp., College Station, TX).

Results

Of 153 MHD patients who underwent EBCT and who had AlkPhos measurements, 16 did not have detectable coronary artery calcification (i.e., CACS = 0). After these patients were excluded, there were 137 MHD patients who had detectable

coronary artery calcification and whose data were analyzed in this study. The 137 patients of the study were 55.3 ± 13.4 yr of age and included 37% women, 34% Hispanic, 46% black, and 60% with diabetes. The mean dialysis vintage was 43 ± 34 mo (median 34 mo; interquartile range 18 to 61 mo). The mean CACS was 1112 ± 2025 (median 416; interquartile range 79 to 1328). The proportions of CACS from 1 to 99, from 100 to 399, from 400 to 999, and ≥ 1000 were 31% ($n = 42$), 19% ($n = 26$), 16% ($n = 22$), and 34% ($n = 47$), respectively.

Table 1 shows the relevant demographic, clinical, and laboratory measures across the three almost equally sized tertiles of CACS with 45 to 47 patients in each tertile. Age, dialysis vintage, and systolic BP were higher in patients in the third CACS tertile. The proportion of patients with diabetes was higher across the worsening CACS tertiles; however, there was no difference across the tertiles in terms of gender, race/ethnicity, body mass index, or biceps and triceps skinfolds. Estimated daily dietary protein and calorie intakes showed a decreasing trend across CACS tertiles, but these trends were not statistically significant. A similarly declining trend was observed for total serum cholesterol and LDL cholesterol in that patients in the lowest CACS tended to have higher LDL cholesterol and *vice versa* ($P = 0.06$ for trend).

There was no trend for serum levels of calcium or phosphorus or their product across the tertiles of CACS. Serum intact PTH showed a declining trend across worsening CACS tertiles (p -trend = 0.053). Compared with PTH, however, serum AlkPhos showed an opposite trend with incrementally higher serum levels across worsening CACS tertiles ($P = 0.08$ for trend). The proportion of patients with serum AlkPhos ≥ 120 IU/L, which was recently suggested as the upper AlkPhos cutoff value for achieving greatest survival in MHD patients (10), was 23, 33, and 45% in the first, second, and third tertiles of CACS, respectively ($P = 0.03$ for trend).

Table 2 shows the correlations between logarithm of CACS and some relevant nutritional, inflammatory, and other biochemical variables. In addition to unadjusted (Pearson) correlation coefficients, multivariate adjusted correlations using linear regression models were calculated to disclose the underlying associations after removing the effect of confounders. Both patient age and dialysis treatment age (vintage) were strong correlates of CACS. Among biochemical values, serum IL-6 and creatinine had significant correlation with CACS, but these associations were not robust to multivariate adjustment. Serum AlkPhos showed a moderate positive correlation with CACS ($r = 0.16$, $P = 0.06$; Figure 1), and the unconfounded association was even stronger after controlling for age, gender, diabetes, liver enzyme (aspartate aminotransferase [AST]), and logarithm of vintage ($r = 0.26$, $P = 0.003$). No other variable resisted multivariate adjustment. To examine further the robustness of the association of serum AlkPhos with CACS, we conducted additional multivariate regression analysis as shown in Table 3. In addition to conventional correlates of increased CACS including age, dialysis vintage, and diabetes, serum AlkPhos was the only biochemical marker with strong association with CACS, especially after multivariate adjustment.

To further examine the association between high AlkPhos

and risk of coronary artery calcification, we dichotomized AlkPhos at 120 IU/L as recently suggested in an epidemiologic study as the cutoff value above which death risk is increased (10). Figure 2 shows that mean total CACS was significantly higher in MHD patients with serum AlkPhos ≥ 120 IU/L compared with < 120 IU/L. This trend was persistent within different coronary arteries, both for number of calcified lesions and for artery-specific CACS (Figure 3). Additional stratified analyses showed that the increase in proportion of patients with AlkPhos > 120 IU/L across increasing tertiles of CACS is robust to confounding by age (Figure 4) as well as dialysis vintage, AST, PTH, minerals, and nutritional or inflammatory markers (data not shown).

Using continuous cubic splines analyses to explore the non-linear association between CACS and AlkPhos, we examined the odds of having a CACS ≥ 400 (the CACS cutoff level above which an extremely high burden of cardiovascular disease and death is reported [18]). As shown in Figure 5, the likelihood of CACS ≥ 400 increased with increasing levels of serum AlkPhos, especially once AlkPhos surpassed 85 IU/L. Table 4 shows the results of unadjusted and multivariate adjusted logistic regression analyses for predicting CACS ≥ 400 as the dependent variable. The unadjusted OR of having CACS ≥ 400 in MHD patients with AlkPhos ≥ 120 IU/L was 3.5 (95% CI 1.5 to 8.3; $P = 0.004$). The OR was robust to multivariate adjustment for other relevant confounders. Indeed, in a fully adjusted model including demographics, comorbidity, IL-6, and AST, the third (highest) AlkPhos tertile (≥ 120 IU/L) was associated with five times higher likelihood of CACS ≥ 400 compared with the middle AlkPhos tertile (85 to 120 IU/L; OR 5.0; 95% CI 1.6 to 16.3; $P = 0.007$). Of note, the lowest tertile of AlkPhos (< 85 IU/L) also showed a trend toward higher risk for coronary artery calcification, which was consistent with the somewhat U-shaped association detected in Figure 5.

Discussion

Examining biochemical markers of nutrition, inflammation, and bone and mineral disorders, we found that serum AlkPhos is the only measure with significant and robust association with the degree of severity of coronary artery calcification in 137 randomly selected MHD patients. Either other biochemical markers had no association with CACS, or their association was eliminated after controlling for case-mix variables, whereas the association between AlkPhos and CACS was robust and even greater after multivariate adjustment. Serum AlkPhos ≥ 120 IU/L was a robust predictor of higher CACS and was particularly associated with the likelihood of CACS ≥ 400 , a cutoff level beyond which significant risk of cardiovascular event and death exists. We also found that a serum AlkPhos of approximately 85 IU/L was associated with the lowest likelihood of severe coronary artery calcification, but a nonsignificant trend with increased CACS was also noticed in the lowest tertile of AlkPhos. Our unprecedented data may have major clinical and public health implications given the high burden of vascular calcification in patients with CKD.

Vascular calcification is usually an important manifestation of significant cardiovascular disease (29). In the general popu-

Table 1. Baseline demographic, clinical, laboratory, and densitometric variables according to tertiles of CACS of 137 MHD patients with CACS >0^a

Variable	CACS			P Trend
	First Tertile (1 to 131; n = 45)	Second Tertile (132 to 1045; n = 45)	Third Tertile (≥1046; n = 47)	
Demographic				
age (yr)	46 ± 14	60 ± 10	60 ± 11	<0.001
women (%)	49	24	38	0.3
black race (%)	51	40	47	0.7
Hispanic ethnicity (%)	36	40	26	0.3
diabetes (%)	36	64	81	<0.001
Nutritional measures				
total proteins	71.6 ± 35.1	68.4 ± 23.4	65.2 ± 28.3	0.3
total calories	1767.0 ± 745.0	1670.0 ± 540.0	1639.0 ± 681.0	0.4
BMI (kg/m ²)	26.5 ± 8.5	25.8 ± 4.4	27.7 ± 5.2	0.4
triceps skinfold (mm)	17.3 ± 10.1	15.1 ± 9.0	16.9 ± 9.2	0.8
biceps skinfold (mm)	10.7 ± 8.5	8.8 ± 6.1	10.0 ± 5.8	0.7
NIR-measured body fat (%)	25.2 ± 12.9	25.0 ± 8.3	28.1 ± 9.7	0.18
Hemodialysis treatment measures				
dialysis vintage <6 mo (%)	7	12	5	0.6
dialysis vintage (mo)	38.0 ± 35.3	37.8 ± 29.7	51.6 ± 35.3	0.01
dialysis dosage (Kt/V single pool)	1.68 ± 0.22	1.71 ± 0.30	1.72 ± 0.28	0.4
nPNA or nPCR (g/kg per d)	1.06 ± 0.19	1.06 ± 0.24	1.12 ± 0.27	0.23
systolic BP (mmHg)	146 ± 28	156 ± 23	157 ± 26	0.06
diastolic BP (mmHg)	79 ± 17	78 ± 15	77 ± 16	0.5
Biochemical measurements (mean ± SD)				
serum albumin (g/dl)	4.03 ± 0.28	3.96 ± 0.28	3.98 ± 0.28	0.4
transthyretin (prealbumin; mg/dl)	31.9 ± 9.7	28.7 ± 8.5	30.1 ± 8.3	0.4
triglycerides (mg/dl)	138 ± 68	144 ± 100	164 ± 165	0.3
TC (mg/dl)	156 ± 43	151 ± 37	145 ± 43	0.22
LDL (mg/dl)	92 ± 37	83 ± 25	79 ± 31	0.06
HDL (mg/dl)	37 ± 9	39 ± 14	34 ± 16	0.3
creatinine (mg/dl)	11.4 ± 3.3	10.2 ± 2.9	10.5 ± 2.9	0.2
ferritin (ng/ml)	568 ± 406	625 ± 475	744 ± 406	0.05
iron saturation ratio (%)	36.1 ± 11.0	35.8 ± 12.1	34.1 ± 10.0	0.4
calcium (mg/dl)	9.8 ± 0.7	9.4 ± 0.6	9.8 ± 0.5	0.6
phosphorus (mg/dl)	5.7 ± 1.2	5.6 ± 1.3	5.5 ± 1.2	0.5
calcium × phosphorus (mg ² /dl ²)	55.7 ± 12.5	52.7 ± 11.5	53.2 ± 11.5	0.3
intact PTH (pg/ml)	349 ± 377	256 ± 188	242 ± 169	0.053
AlkPhos (IU/L)	104 ± 51	120 ± 59	126 ± 56	0.08
AlkPhos ≥120 IU/L (%)	23	33	45	0.03
serum AST (U/L)	17.9 ± 11.2	20.3 ± 18.5	18.4 ± 7.7	0.9
bicarbonate (mg/dl)	22.3 ± 2.8	22.4 ± 3.0	22.6 ± 3.1	0.6
total homocysteine (μmol/L)	26.0 ± 8.3	26.0 ± 9.8	27.5 ± 8.3	0.4
CRP (mg/l)	5.5 ± 6.5	4.7 ± 4.8	4.5 ± 4.4	0.9
IL-6 (pg/ml)	22.4 ± 95.5	7.9 ± 5.4	11.0 ± 8.4	0.04
TNF-α (pg/ml)	6.2 ± 9.9	5.8 ± 5.8	6.4 ± 5.7	0.4
blood hemoglobin (g/dl)	12.2 ± 0.7	12.5 ± 0.7	12.1 ± 0.7	0.3
WBC (×1000 cell/μl)	6.6 ± 2.1	7.0 ± 1.6	6.8 ± 1.5	0.6
lymphocyte (% of total WBC)	28.3 ± 8.4	23.5 ± 6.1	22.5 ± 7.8	<0.001
Medications				
erythropoietin dosage (1000 U/wk)	11.4 ± 7.0	10.0 ± 11.2	13.6 ± 1.1	0.3
paricalcitol dosage (mg/mo)	69 ± 83	41 ± 25	49 ± 53	0.17

^aData are means ± SD or percentages. P values for dialysis dosage (vintage), ferritin, CRP, IL-6, and TNF-α are based on the logarithmic values of these measures. AlkPhos, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; CACS, coronary artery calcification score; CRP, C-reactive protein; Kt/V, dialysis dosage; MHD, maintenance hemodialysis; NIR, near-infrared; nPCR, normalized protein catabolic rate; PTH, parathyroid hormone; TC, total cholesterol; WBC, white blood cell.

Table 2. Bivariate (unadjusted) and partial (adjusted) correlation coefficients between logarithm of CACS and relevant variables of 137 MHD patients with CACS >0^a

Variable	Bivariate Correlation	P	Adjusted Correlation	P
Age	0.49	<0.001	0.39	<0.001
Dialysis vintage (log scale)	0.16	0.06	0.33	<0.001
BMI	0.06	0.5	0.05	0.6
NIR body fat percentage	0.13	0.13	0.04	0.6
Dietary protein intake	-0.11	0.21	-0.04	0.7
Dietary energy (calorie) intake	-0.10	0.3	0.06	0.5
Serum calcium	-0.05	0.6	0.04	0.7
Phosphorous	-0.10	0.3	0.12	0.2
Calcium × phosphorous	-0.12	0.2	0.12	0.2
Intact PTH (log scale)	-0.14	0.11	0.11	0.2
AlkPhos	0.16	0.06	0.26	0.003
AST (log scale)	0.08	0.4	0.03	0.7
Albumin	-0.06	0.5	0.04	0.7
Transferrin	-0.03	0.7	0.04	0.7
TIBC	0.02	0.8	-0.03	0.8
Ferritin	0.14	0.1	0.08	0.4
Bicarbonate	0.03	0.7	0.06	0.5
Creatinine	-0.20	0.02	-0.02	0.8
IL-6 (log scale)	0.22	0.01	0.1	0.3
TNF- α	0.09	0.3	0.14	0.12
CRP	-0.01	0.9	0.01	0.9
LDL	-0.09	0.3	-0.02	0.8
HDL	-0.01	0.9	0.02	0.8
TC	0.02	0.3	0.02	0.8
Blood hemoglobin	-0.05	0.6	0.01	>0.9
WBC	0.09	0.3	0.002	>0.9
Lymphocytes %	-0.29	<0.001	-0.13	0.2
Paricalcitol dosage	-0.11	0.3	-0.08	0.5

^aIn the adjusted analysis, age, gender, diabetes, AST, and log vintage were included as covariates. TIBC, total iron-binding capacity.

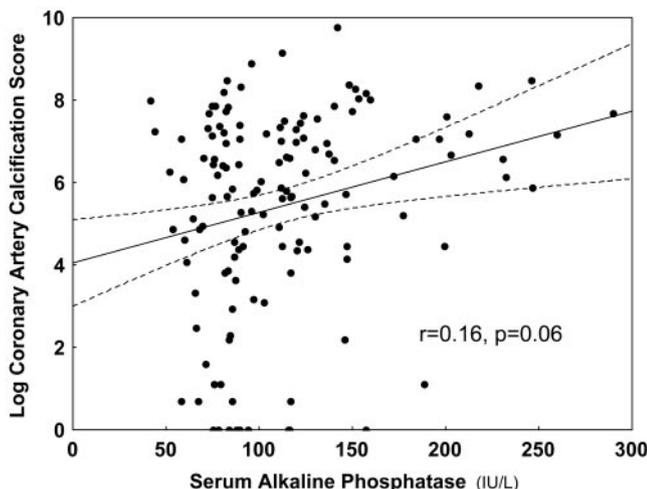


Figure 1. Scatter plots, regression line, and 95% confidence intervals, reflecting the correlation between serum alkaline phosphatase (AlkPhos) and logarithm of coronary artery calcification score (CACS).

Table 3. Multiple regression models to predict log CACS of 137 MHD patients

Parameter	$\beta \pm SE$	P
Unadjusted		
AlkPhos (IU/L)	0.007 \pm 0.004	0.06
Adjusted		
age (yr)	0.070 \pm 0.010	<0.001
gender (male)	0.440 \pm 0.350	0.22
diabetes	1.650 \pm 0.410	<0.001
log vintage (mo)	0.740 \pm 0.180	<0.001
log AST (IU/L)	-0.050 \pm 0.380	0.90
log IL-6 (ng/ml)	0.240 \pm 0.220	0.30
AlkPhos (IU/L)	0.010 \pm 0.003	0.003

lation, the severity score of coronary artery calcification obtained *via* EBCT was shown to be a strong and robust predictor of cardiovascular events and death when compared with con-

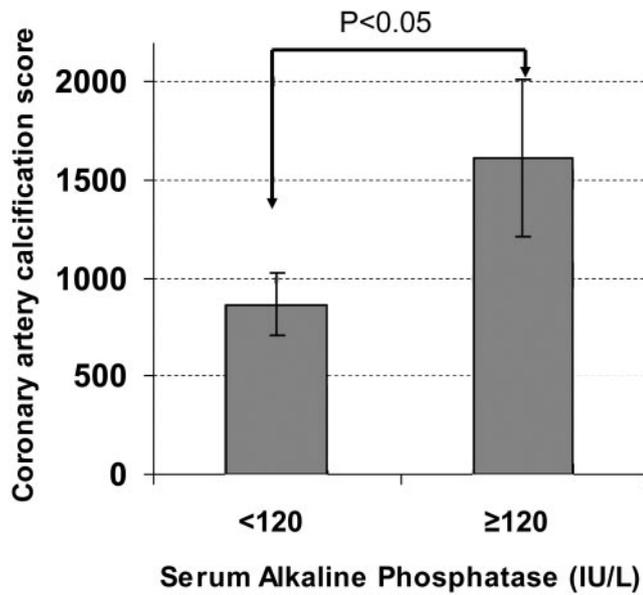


Figure 2. Comparison of total CACS and the number of calcified lesions in maintenance hemodialysis (MHD) patients with serum AlkPhos < and ≥120 IU/L (error bars represent SEM).

ventional cardiovascular risk factors (30). In the patient population with CKD, coronary artery calcification is extremely common, especially among those who have been undergoing dialysis treatment for a longer period of time (2). In our study, almost 90% of all MHD patients had detectable coronary calcification; only 16 of the 153 MHD patients who randomly underwent EBCT had CACS = 0. Some but not all studies that have examined the potential risk factors of coronary artery calcification among patients with CKD have implicated both the conventional risk factors such as diabetes (31) and dyslipidemia (32,33) and CKD-specific factors such as inflammation (34–37), fetuin deficiency (38,39), SHPT (40), hypercalcemia or high calcium load (41), and high calcium-phosphorus product (42); however, there have been contradictory data about the

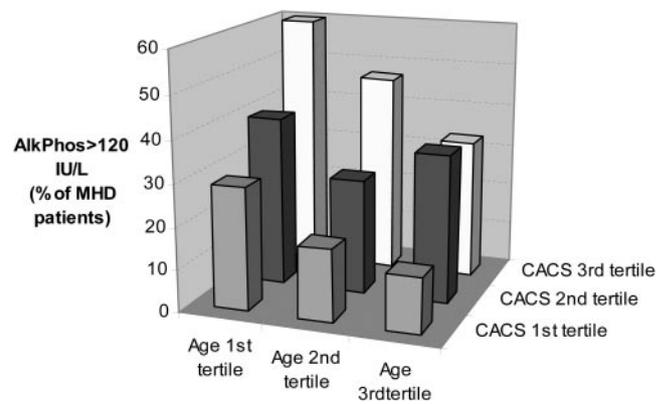


Figure 4. Proportion of MHD patients with an AlkPhos >120 IU/L in tertiles of age and CACS.

association of some of these factors and vascular calcification. One study did not find any associations between blood levels of minerals or PTH and CACS in nondialyzed patients with CKD (43). Similarly, in our study, minerals or PTH did not correlate with CACS, but AlkPhos did. Indeed, there was a negative trend between serum intact PTH and CACS. Inflammatory markers including IL-6 did not show an independent association with CACS either.

Consistent with the findings of our clinical study, there are recent *in vitro* findings about the association of AlkPhos and vascular calcification. Lomashvili *et al.* (5,7) reported that expression of tissue-nonspecific AlkPhos in the setting of vascular damage can lead to hydrolysis and inactivation of inorganic pyrophosphates. Inorganic pyrophosphates are potent inhibitors of hydroxyapatite crystal growth and a potential local and circulating inhibitor of vascular calcification (44). In animal models, vascular calcification can be induced by lowering of the levels of inorganic pyrophosphate (44). Animal studies have also shown that under uremic conditions, AlkPhos is upregulated in vessels (7).

Bone-specific AlkPhos is increased in MHD patients, proba-

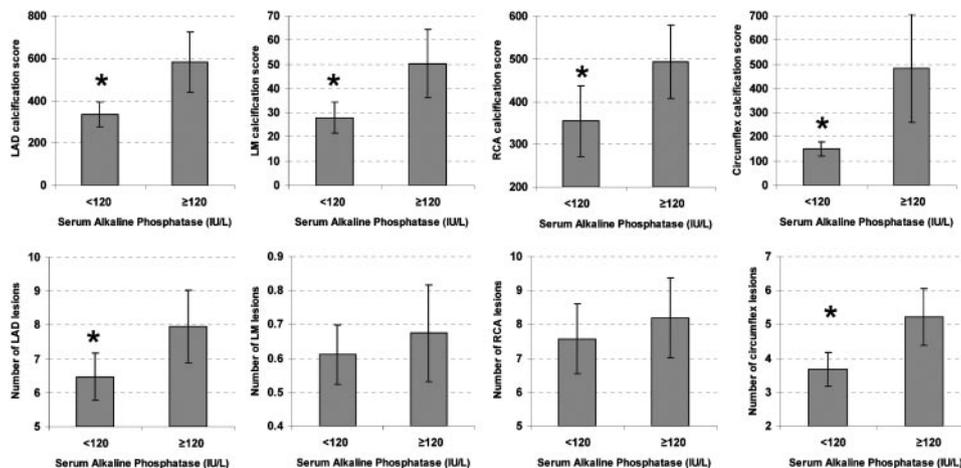


Figure 3. CACS and the frequency of calcified lesions in the four coronary arteries in MHD patients with serum AlkPhos < and ≥120 IU/L. LAD, left anterior descending; LM, left main; RCA, right coronary artery. *P < 0.005. Error bars represent SEM.

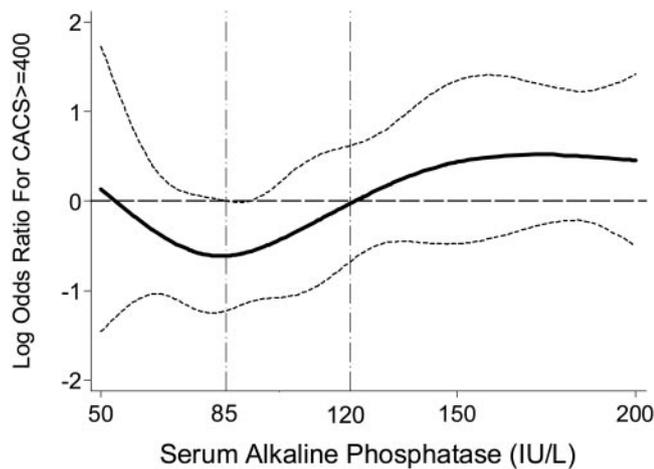


Figure 5. Log odds ratio of having CACS \geq 400 across spectrum of serum AlkPhos in 137 MHD patients.

bly as a result of high-turnover bone disease (45); indeed, a statistical association between bone AlkPhos level and the presence of aortic calcification is present in patients with osteoporosis (46). Similarly, an *in vivo* study showed an increased level of circulating bone-specific AlkPhos in patients with CKD in the presence of aortic calcification (45). Even though active vitamin D analogs including calcitriol may directly modulate calcifying processes leading to decreased vascular calcification, their salutary affects may also be mediated *via* osteopontin and AlkPhos pathways (6). A recent epidemiologic study of >70,000 MHD patients showed that an increased level of AlkPhos was associated with all-cause and cardiovascular mortality (10). In that study, a serum AlkPhos >120 IU/L was associated with significant death risk across almost all subgroups of MHD patients (10). The discovered association between AlkPhos and CACS in MHD patients in our study provides a

biologically plausible link between such epidemiologic data (10,47,48) and the *in vitro* studies that link AlkPhos to vascular calcification *via* modulation of the pyrophosphate pathway (5,7).

Our study should be qualified for a number of limitations, including selection bias during enrollment, leading to younger MHD patients, who tended to have higher serum AlkPhos (Figure 4); however, because mortality is lower in younger MHD patients (13), it might be argued that a selection bias with such a direction generally would lead to a bias toward the null, so without this bias, our positive results might have been even stronger. Another limitation of our study is that we did not have bone biopsy specimens to determine histologic assessment of osteodystrophy. Furthermore, liver disorders, which are not accounted for in our analyses, may lead to increased AlkPhos and confound the examined associations. Subgroup analyses for phosphorus binder types were not performed, because >85% of our patients received sevelamer hydrochloride. Even though such SHPT therapies may lead to an initial surge in serum AlkPhos (41,49), the level will eventually fall with improvement of SHPT; we used 3-mo averaged AlkPhos values rather than one single AlkPhos measurement to mitigate confounding by such short-term surges. Notwithstanding these limitations, the strengths of our study include the moderate sample size, the comprehensive clinical and laboratory evaluations including body composition measures, detailed evaluation of comorbid states by study physicians, and measurement of proinflammatory cytokines.

Conclusions

In our study, serum AlkPhos was the only biochemical measure with independent association with coronary artery calcification in 137 randomly selected MHD patients. Serum AlkPhos \geq 120 IU/L was associated with the likelihood of CACS

Table 4. Unadjusted and adjusted OR (95% CI) of having CACS \geq 400 across tertiles of serum AlkPhos in 137 MHD patients^a

Parameter	Serum AlkPhos (IU/L)				
	First Tertile (<85; n = 45)		Second Tertile (85 to 119; n = 46)		Third Tertile (\geq 120; n = 46)
	OR (95% CI)	P	OR (95% CI)	OR (95% CI)	P
Unadjusted ORs	1.96 (0.84 to 4.55)	0.120	1.00 (reference)	3.52 (1.49 to 8.29)	0.004
Adjusted ORs					
age	2.22 (0.89 to 5.54)	0.090	1.00 (reference)	4.63 (1.79 to 11.98)	0.002
age + gender	2.27 (0.90 to 5.74)	0.080	1.00 (reference)	5.98 (2.16 to 16.58)	0.001
age + gender + diabetes	2.43 (0.93 to 6.37)	0.070	1.00 (reference)	6.21 (2.17 to 17.76)	0.001
age + gender + diabetes + Charlson	2.89 (1.05 to 7.96)	0.040	1.00 (reference)	6.03 (2.09 to 17.39)	0.001
age + gender + diabetes + Charlson + vintage	3.01 (1.01 to 8.99)	0.050	1.00 (reference)	5.75 (1.84 to 18.02)	0.003
age + gender + diabetes + Charlson + vintage + IL-6 + AST	2.41 (0.79 to 7.39)	0.120	1.00 (reference)	5.03 (1.55 to 16.34)	0.007

^aLog-transformed value of dialysis vintage, IL-6, and AST were used in these analyses. CI, confidence interval; OR, odds ratio.

≥400. Our findings may have major clinical and public health implications given the high burden of vascular calcification in patients with CKD and potential therapeutic strategies to modulate this pathway to mitigate or prevent risk for vascular calcification and improve the poor survival of patients with CKD.

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