Original Paper

Classification of Chronic Kidney Disease Biomarkers to Predict Coronary Artery Calcium

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Key Words

Serum phosphorus • Estimated glomerular filtration rate • Chronic kidney disease • Coronary artery calcification • Growth mixture modeling • Two-part growth modeling

Abstract

Background/Aims: The link between CKD and CAC has been mostly established by studies of patients who have abnormally high phosphorus levels and advanced CKD or end-stage renal disease. The aim of this study was to examine if there are distinct trajectory classes of serum phosphorus (controlling for eGFR) that are associated CAC in a relatively healthy, community sample. **Methods:** Phosphorus and eGFR were classified as a combined biomarker variable with 4 trajectory classes by growth mixture modeling. This classification variable was subsequently used to predict CAC as both a binary (i.e., onset) and continuous (i.e., accumulation) outcome using a two-part growth model. **Results:** Membership in one class of phosphorus trajectory versus the next lowest level was associated with a 97.9 Agatston unit increase in CAC ($p < .001$). The magnitude of this finding is similar in size as some primary risk factors for cardiovascular disease, including a 55.3 Agatston unit ($p < .001$) increase associated with age, and a - 75.1 Agatston unit ($p < .001$) decrease associated with female gender. **Conclusions:** Classification of phosphorus trajectories provides further definition for prediction of CAC within the conventional 'normal' range. Classifying trajectories may help determine clinically-relevant thresholds for interventions aimed at phosphorus reduction.

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Introduction

The link between CKD and CAC has been mostly established by studies of patients who have abnormally high phosphorus levels and advanced CKD or end-stage renal disease (ESRD) [1-8]. However, smaller elevations in phosphorus and development of CAC may occur in early-stage, or even subclinical, CKD. One study reported a strong connection between eGFR, phosphorus, and CAC within a community-based sample, even though most participants did not have abnormal eGFR or phosphorus levels [4]. In another community-based cohort, the Spokane Heart Study (SHS), we found a robust relationship between changes in the serum phosphorus level and CAC over time that was similar in magnitude to that of traditional cardiovascular disease (CVD) risk factors [9]. Notably, experimental models support biologically-plausible mechanisms for hyperphosphatemia to induce CAC by promoting vascular tissue calcification [2, 7, 10, 11]. Yet understanding remains vague regarding the complex inter-relationships between serum phosphorus, kidney function, and CAC.

The primary aim of this study was to examine whether phosphorus classes predict CAC among participants in the SHS. The statistical technique of growth mixture modeling allows pre-specification of likely 'classes' (i.e., sub-groups) of a predictor variable to explain heterogeneity in change of an outcome variable over time as has been demonstrated for conditions such as obesity, depression and smoking [12-14]. After auditioning various multi-class solutions and identifying an optimal number of classes, we then used this classification variable to predict CAC in a two-part latent growth model. This type of longitudinal CAC model treats CAC as both binary and continuous given its semicontinuous nature [15], as has been demonstrated in other clinical domains [16, 17].

Materials and Methods

Study design and participants

The SHS is a longitudinal cohort study of a community-based sample, originally intended to identify non-traditional risk factors for CVD. The first patient was enrolled in the SHS in April, 1994. Participants were asked to return for a follow-up visit every two years until 2004. Thus, the maximum number of visits that a participant could have had during active data collection was six. The sample consists of both men and women above the age of 18 years who were employed in various fields that include firefighting, law enforcement, healthcare, education, and business. Subjects who were enrolled were believed to be in good health without known CVD or other illnesses associated with major co-morbidities or a reduced life expectancy. All subjects provided written informed consent to enter the study, which was reviewed and approved by the Washington State University Institutional Review Board (IRB) and the Spokane-IRB, which oversee protection of human research subjects in the community. Participants in the present dataset were above the age of 30 years and had at least 2 visits on a biennial basis.

Variables

At each study visit, electron beam computed tomography (EBCT) was performed to measure CAC. Specifically, CAC was quantified by pixel density in a gated scan with 3 mm image slices. This density was converted to Hounsfield units, and each artery's score was totaled for an overall CAC score in Agatston units [3]. In addition, blood and urine samples were collected and analyzed at a central laboratory (Pathology Associates Medical Laboratory, Spokane, WA). At each assessment, blood was collected in a fasting state. Standard clinical serum chemistries and hematology were performed on all samples. Subjects also filled out an extensive questionnaire, which included questions about lifestyle, habits, medical history, and various psychological measures.

Variables used in the analyses include age, gender, diastolic blood pressure (DBP), systolic blood pressure (SBP), diabetes status, smoking status, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol (TC), triglycerides, body mass index (BMI), eGFR (using the re-expressed Modification of Diet in Renal Disease equation, MDRD), and serum phosphorus (see Table 1). A positive diabetes status was defined as having a fasting glucose value ≥ 126 mg/dl on at least two study visits. The serum creatinine measurements were aligned with a nationally representative sample (NHANES, National Health and Nutrition Education Survey) in order to reliably compute eGFR with the re-expressed

Table 1. Baseline characteristics of participants in the Spokane Heart Study from 1994-2004 (n=721).

	Mean n/N	+/- SD %
Age (year)	49.0	9.2
DBP (mm Hg)	78.8	16.4
SBP (mm Hg)	123.7	16.0
HDL cholesterol (mg/dl)	53.8	15.8
LDL cholesterol (mg/dl)	132.7	33.9
TC (mg/dl)	215.1	40.9
Phosphorus (mg/dl)	3.3	0.5
eGFR (ml/min/1.73m ²)	112.3	41.2
BMI (kg/m ²)	27.0	4.5
Triglycerides (mg/dl)	145.6	104.2
CAC (Agatston units)	60.8	195.7
Glucose (mg/dl)	91.7	11.3
White race	692/721	96
Female	288/721	43
Diabetes	94/721	13
Smoke (current versus ever)	144/721	20
Note: DBP = diastolic blood pressure; SBP = systolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TC = total cholesterol; BMI = body mass index.		

MDRD equation, as was previously reported in analyses of this database [18]. The serum creatinine values obtained in the SHS were reduced by 0.27 mg/dl in order to reconcile with the standardized creatinine levels based on NHANES data [9].

Statistical analyses

Paired sample t-tests were used to test for differences between baseline and the final study visit on DBP, SBP, HDL cholesterol, LDL cholesterol, TC, serum phosphorus, eGFR, BMI, triglycerides, and glucose, but all other analyses utilized latent variable techniques. Latent variable modeling is a class of statistical techniques that includes both finite growth mixtures and two-part latent growth curve models. First, a standard growth model was fit to the sample trajectory of phosphorus over time, while controlling for the impact of eGFR as a time-varying covariate (data not shown). Second, the subsequent analysis divided heterogeneous trajectories into several classes that shared a statistically homogenous trajectory shape, a method known as finite growth mixture modeling [19] that has been applied in other clinical research domains [12-14, 17]. In the third and final analysis, this new classification variable was used to predict CAC in a growth model of CAC. The CAC two-part growth model accounted for the probability of onset at study entry and over time (i.e., CAC present or absent at each visit), and the amount of CAC accumulation at study entry and change over time (i.e., CAC on a scale from 1-3000+) [15]. Log-transformed CAC (ln (CAC)) was used due to severe skewness, and is a typical transformation that is required for two-part latent growth models and in the statistical treatment of CAC [11]. The complete two-part model with random growth factors regressed on the 11 predictors are displayed in Fig. 1.

Initial analyses were conducted using Stata 11.0 (StatCorp, 2009, www.stata.com) followed by latent variable analyses with Mplus 5.21 (Muthen & Muthen, 2009, www.statmodel.com). This approach managed missing data with the use of the full information maximum likelihood and the robust maximum likelihood estimator, to allow for violations of normality. We used established model fit criteria (i.e., Bayesian Information Criteria, Bootstrapped Likelihood Ratio Test) to choose the most parsimonious model [20].

We estimated a 1- though 6-class solution for a thorough comparison of serum phosphorus trajectory classes (controlling for the time-varying effect of eGFR). Based on the Bootstrapped Likelihood Ratio Test, Bayesian Information Criteria, and Log Likelihood, the 4 class model was chosen [20]. The mean amount of

Fig. 1. Conditional two-part latent growth curve model of CAC with both traditional and non-traditional covariates for participants in the Spokane Heart Study from 1994-2004. Please note that while all path coefficients and exogenous covariate covariances are shown in the figure, not all path and covariances were estimated in the final model. In this model diagram, one can see all of the visit-specific scores for the binary version of CAC (bottom row of rectangle boxes) and the continuous version of CAC (top row of rectangle boxes). The growth factors (i.e., random effects) of CAC change over time are represented in the ovals labeled Intercept, Slope, and Quadratic. The beta coefficients and corresponding arrows going to the ovals represent the prediction of CAC change over time by the 11 covariates listed in the far left rectangle box and presented formally in Table 3. All of the other terms presented in the model represent error terms, or unique variance unaccounted for by the specified model. Allowance of uniqueness terms and covariances between the covariates of interest is a unique feature of this type of modeling strategy.

phosphorus at baseline and over time varied across classes (see Table 2). The average posterior probability (i.e., a measure from 0 to 1 of individuals being correctly classified, 1 indicating that 100% of the individuals were correctly classified) was 0.85, which is an indication that the four classes in Table 2 and Fig. 2 were well defined.

For the two-part growth model, CAC was regressed as a binary outcome (i.e., onset, or presence/absence of CAC) and as a continuous outcome (ln (CAC); accumulation) on the pre-specified covariates simultaneously. Each measure of CAC was modeled over time and using predictor variables to account for

Table 2. Mean intercept and slope by phosphorus class of participants in the Spokane Heart Study from 1994-2004 (n=721).

	Low (11.6%)	Normative (44.7%)	Elevated (39.1%)	Highly Elevated (4.6%)
Mean Intercept (SE)	2.5 (0.15)	3.0 (0.1)	3.5 (0.1)	4.0 (0.2)
Mean Slope (SE)	0.2 (0.04)	0.1 (0.0)	0.1 (0.0)	0.1 (0.1)

Note: SE = standard error.

Fig. 2. 4-Class solution of finite growth mixture model means plotted over 6 years for participants in the Spokane Heart Study from 1994-2004.

variation in baseline (intercept) and change over time (slope). Through the process of iterative model fitting, paths were eliminated to obtain a model specification that would converge [8] and produced adequate model fit.

Results

Participants in this analysis (n = 721) were between the ages of 30 and 86 years with a mean (+/- SD) of 49 +/- 9 years; 43% (288/721) were female, 96% (692/721) were of white race, and the mean BMI was 27.0, which has generally been considered to be in the 'overweight', but not 'obese' category [14] (Table 1). 65% (467/721) of the sample had a CAC score of 0 at baseline, and an additional 23% (165/721) had a CAC score < 100. Approximately six years after the baseline assessment of the SHS participants, there was a significant decrease in eGFR from 112.25 +/- 41.16 ml/min/1.73m² at baseline to 102.01 +/- 23.79 ml/min/1.73m² 6 years later (t = 4.75, df = 741.77, p < 0.001) and a significant increase in glucose from 91.71 +/- 11.25 mg/dl at baseline to 95.39 +/- 12.4 mg/dl (t = -4.11, df = 392.57, p < 0.001). No other variables demonstrated a significant change from baseline to the final visit.

Table 3. Covariate effects on the two-part latent growth model of CAC for participants in the Spokane Heart Study from 1994-2004 (n=721).

	CAC Onset at Baseline (Positive CAC at Study Entry)			CAC Accumulation at Baseline (Amount of CAC at Study Entry)		
	β (95% CI)	Z-test	P	β (95% CI)	Z-test	P
Phosphorus Class	0.26 (0.14, 0.38)	4.1	< 0.001	0.50 (0.38, 0.61)	8.6	< 0.001
Age (year)	0.28 (0.16, 0.40)	4.6	< 0.001	0.00 (0.00, 0.00)	---	---
Female	- 0.35 (- 0.45, -0.25)	- 6.8	< 0.001	- 0.33 (-0.44, - 0.23)	- 6.2	< 0.001
Smoke (current versus ever)	0.03 (-0.11, 0.17)	0.1	0.67	0.00 (0.00, 0.00)	---	---
HDL cholesterol (mg/dl)	- 0.03 (- 0.14, 0.07)	- 0.6	0.55	0.01 (- 0.14, 0.07)	0.1	0.93
LDL cholesterol (mg/dl)	0.10 (0.12, 0.19)	2.2	0.03	0.03 (- 0.06, 0.12)	0.7	0.50
Triglycerides (mg/dl)	0.09 (0.01, 0.17)	2.2	0.03	0.16 (0.06, 0.26)	3.3	0.001
SBP (mm Hg)	0.06 (- 0.05, 0.17)	1.1	0.26	0.17 (0.02, 0.31)	2.3	0.02
DBP (mm Hg)	- 0.02 (- 0.11, 0.07)	- 0.5	0.61	- 0.14 (- 0.37, 0.08)	- 1.2	0.22
BMI (kg/m ²)	- 0.01 (- 0.11, 0.08)	- 0.3	0.77	- 0.02 (- 0.12, 0.07)	- 0.5	0.62

Note: β = linear regression coefficients; DBP = diastolic blood pressure; SBP = systolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; BMI = body mass index

The growth mixture analysis revealed distinct patterns that represent four trajectory classes of individuals: Highly Elevated (n = 33), Elevated (n = 282), Normative (n = 322), and Low (n = 84) (Fig. 2, Table 2). The Highly Elevated group began with a mean phosphorus level of 3.97 mg/dl at study entry, and increased 0.14 mg/dl every two years. The Elevated group had a mean of 3.46 mg/dl at study entry, and increased 0.10 mg/dl every two years. The Normative group began the study with a mean of 3.02 mg/dl and showed an increase of 0.10 mg/dl every 2 years thereafter. Finally, the Low group had a mean of 2.53 mg/dl at the start of the study, and increased 0.16 mg/dl every two years. The resulting class membership variable was used in the next analysis as a predictor of CAC onset and accumulation.

We estimated a two-part latent growth model for CAC. There was a significant correlation between the CAC accumulation intercept and slope ($r = - 0.38$, $p < 0.001$) such that the lower the CAC score at baseline, the more CAC increased over time. CAC onset (intercept) and accumulation (intercept and slope) was regressed on the covariates (i.e., age, female gender, diabetes status, smoking status, HDL, LDL, DBP, SBP, triglycerides and BMI). The variability of change in the probability of a positive scan across 4 visits (onset slope), and the quadratic variability of change for $\ln$ (CAC) was non-significant. Thus, the amount of both change in the probability of getting a positive scan and quadratic change in $\ln$ (CAC) was not estimated (Fig. 1).

Table 3 contains the linear regression results of our pre-determined covariates predicting CAC onset and accumulation. Membership in one class of phosphorus trajectory versus the next lowest level predicted a 97.8 Agatston unit increase in CAC accumulation ($\beta = 0.50$, $p < 0.001$) and a significant increase in the probability of onset at baseline ($\beta = 0.26$, $p < 0.001$) while controlling for 10 other, traditional risk factors (Fig. 3). A 104 mg/dl (one SD) increase in triglyceride level was also associated with an increase in the probability of CAC onset ($\beta = 0.09$, $p = 0.03$) and an increase of 31.3 Agatston units of CAC accumulation ($\beta = 0.16$, $p < 0.001$) at baseline, while smoking was the only variable associated with an increase of 74.4 Agatston units of CAC accumulation from visit to visit ($\beta = 0.38$, $p < 0.001$). A 9.6 year (one SD) increase in age was also positively associated with the probability of CAC onset at

Fig. 3. Median CAC score over 6 years by phosphorus class for participants in the Spokane Heart Study from 1994-2004.

baseline ($\beta = 0.28$, $p < 0.001$), and female gender was negatively associated CAC onset ($\beta = -0.35$, $p < 0.001$) and with a lower level of CAC accumulation at baseline (-64.6 Agatston units, $\beta = -0.33$, $p < 0.001$). An LDL increase of 33.9 mg/dl (one SD) was positively related to the probability of CAC onset at baseline ($\beta = 0.10$, $p = 0.02$), while a 16 mm Hg (one SD) increase in SBP was associated with a 33.3 Agatston unit increase in CAC accumulation at baseline ($\beta = 0.17$, $p = 0.02$). The amount of variance accounted for in CAC, both onset and accumulation, was uniformly high for all repeated measures of CAC (i.e., $> 92\%$). There was no effect of HDL cholesterol or SBP, and despite the sample having a mean BMI of 27.0 at baseline, BMI was not predictive of CAC.

Discussion

Phosphorus class was predictive of presence and amount of CAC at baseline, which predicted the increase in CAC over time given the significant intercept and slope relationship. Four significant classes of phosphorus trajectories across 6 years were observed among a healthy cohort that was largely free of CKD at baseline. Although levels were within the conventional “normal range,” these classes could be described as a relatively low level of phosphorus, a normative class with an average amount of phosphorus, a class with an elevated level of phosphorus, and a class with a highly elevated level of phosphorus. In addition to other predictors, this newly created classification variable for phosphorus was shown to be a primary predictor of CAC presence and amount at study entry controlling for the impact of time-varying kidney function. CAC has been notoriously difficult to appropriately analyze [21] and this study demonstrates a potential alternative for analyzing CAC. It is also important to recognize that simultaneously modeling CAC as both an onset and accumulation variable allowed these predictor variables to exert differential impact on CAC.

The current investigation provides new knowledge about the longitudinal association between serum phosphorus and CAC [9]. This statistically-derived classification system provides potential cut-points for phosphorus that may be used to indicate relevant target levels (i.e., elevated and highly-elevated) for potential phosphorus-reducing interventions. In addition, the current investigation shows that the way CAC is defined, (i.e., as a binary

or continuous variable), makes a significant difference in how identified covariates of CAC express themselves. Importantly, previous studies have only investigated CAC as either a binary or a continuous variable, but not both [21]. The present study demonstrates that the influence of CVD risk factors on CAC depends to some extent on which method of analysis is used. These data also support previous observations that elevation of serum phosphorus within the conventional normal range is a risk factor for CAC onset and progression [5, 22].

Mechanisms that underlie the association between phosphorus and vascular calcification are likely multi-factorial. For example, phosphorus induces vascular smooth muscle cells to transform to an osteoblastic phenotype which promotes mineralization of extracellular matrix and deposition of arterial calcium [2, 7, 10, 11]. Genetic mechanisms may contribute to this tendency by altering expression of phosphorus regulating factors. In non-uremic rodents, fibroblast growth factor-23 (FGF₂₃) or Klotho deficiency raise serum phosphorus and cause vascular calcification [23, 24]. Interestingly, smoking was the only significant covariate of CAC growth over time (i.e., rate of CAC accumulation over time), suggesting that this environmental risk factor could exacerbate a predisposition for increasing phosphorus.

Clinical trial evidence with adult hemodialysis patients suggests that using non-calcium-based phosphate binders is associated with significantly less progression of CAC than using calcium-based binders in advanced CKD [1]. In a recent study of patients receiving dialysis treatment, patients 65 years or older who received a non-calcium-based phosphate binder (sevelamer) compared to calcium-based phosphate binders were at lower risk of death and experienced fewer hospitalizations [25]. This is a key finding because treating patients with calcium-based phosphate binders at advanced stages of CKD may contribute to greater CAC accumulation in patients who are already at considerable risk for CVD [6, 26].

This study has several limitations worthy of note. First, the classification strategy of finite mixture modeling is largely exploratory because there is no previous research that has established a classification for serum phosphorus trajectories. Second, the relatively modest size of the sample that mostly included individuals with normal kidney function is a limitation, but these features would be expected to reduce strength of the associations. Consequently, actual relationships could be more robust than reported here. Third, neither c-reactive protein nor FGF₂₃ were available for analysis in our dataset, but we were able to control for most other traditional and many other non-traditional risk factors. Future follow-up research should consider including c-reactive protein and FGF₂₃ in analyses. Finally, the lack of discernable effect of phosphorus class on the CAC slope may reflect a timeframe that is too short (6 years) to detect the long chronicity of CAC accumulation. Nevertheless, given the empirical strength of this novel statistical model, the phosphorus classification scheme is worthy of validation in other cohorts including those with and without CKD.

In conclusion, the higher the class of phosphorus trajectory – even within the conventional normal range – the greater is the probability of CAC presence and increased severity independent of kidney function. The distinct patterns of phosphorus trajectory could be used to inform studies of phosphorus-lowering treatments designed to reduce accumulation of CAC. This novel statistical conceptualization also provides evidence that CVD risk factors operate differently, depending on whether CAC is characterized as a binary or continuous variable.

Conflict of Interest Statement

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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