Calcification or Classification?

Glenn M. Chertow* and Sharon M. Moe†

*Division of Nephrology, Departments of Medicine, Epidemiology, and Biostatistics, University of California San Francisco, California; †Division of Nephrology, Department of Medicine, Indiana University, Indianapolis, Indiana

J Am Soc Nephrol 16: 293–295, 2005. doi: 10.1681/ASN.2004121115

In this issue of *JASN*, Kestenbaum and colleagues (1) report the results of a large epidemiologic inquiry focused on risks associated with serum phosphorus in predominantly male veterans with chronic kidney disease (CKD). More than 95,000 veterans were identified with two or more serum creatinine (SCr) determinations over a 3-yr period; 6730 met the authors' definition of CKD (excluding ESRD) and 3490 had one or more determinations of serum phosphorus. The authors' primary analysis was time to death using the proportional hazards model, with at least 13 months of follow-up. Models were adjusted for age, sex, race, diabetes, cardiovascular diagnoses, hemoglobin, serum calcium, and a variety of estimates of kidney function. Relative to individuals with serum phosphorus concentrations between 2.5 and 3.0 mg/dl, individuals with serum phosphorus concentrations between 3.5 and 4.5 mg/dl experienced a >30% increase in the risk of death; serum phosphorus concentrations of 4.5 or above were associated with a >80% increase in risk. The higher risk associated with higher serum phosphorus was not extinguished in companion models where additional laboratory values and the use of selected medications were considered. The authors suggested that serum phosphorus might exert a direct adverse effect in patients with CKD by enhancing vascular calcification, citing experimental evidence that demonstrated calcification in vascular smooth muscle cells (VSMC) exposed to media with inorganic phosphate concentrations of 1.4 mmol/L (approximately 4.3 mg/dl) (2). Hyperparathyroidism and 1,25 dihydroxyvitamin D deficiency associated with hyperphosphatemia were also noted as potential mediators.

Validity of the Results

As with all epidemiologic analyses, validity depends on the quality of the data, appropriateness of the analysis (including handling of missing data), careful consideration of confounding variables, and biologic plausibility. The US Department of Veterans Affairs (VA) hospitals and health systems have been among the leading institutions in computerized medical records; data from other VA studies using administrative and other electronic records have been exceptionally informative. Data quality from the Consumer Health Information and Performance Sets (CHIPS) is likely to be high.

Conventional survival techniques were used for most of the analyses. We were troubled with the authors' inferences regarding exposures up to three years before survival was evaluated. However, if the exposure period were narrowed, the absolute number and fraction of individuals with serum phosphorus data would have been lower, reducing generalizability as well as sample size and statistical power. Mortality rates were 25% higher for veterans with CKD who had serum phosphorus measured compared with those who did not, although the mean estimated creatinine clearance (eCrCl) of both groups was nearly identical. This suggests that the analytic sample was biased relative to all veterans with CKD.

Multiple confounding variables were considered and adjusted for. While certain comorbid conditions might be underascertained in some electronic datasets relative to direct patient inquiry or medical records review, a deficiency here is unlikely to qualitatively influence the results. There were no obvious omissions, *i.e.*, variables strongly associated with serum phosphorus that were not considered. Parathyroid hormone concentrations were obtained in only 7% of veterans with CKD and a value for serum phosphorus; the number of veterans with available 25-hydroxyvitamin D concentrations was likely even lower. These findings confirm results from previous studies (3) and highlight the fact that relevant disorders of mineral metabolism are generally not investigated in persons with mild to moderate CKD.

Several epidemiologic studies have demonstrated an association between higher serum phosphorus, mortality, and cardiovascular disease in patients on hemodialysis. Pooling two random samples of prevalent US hemodialysis patients evaluated during the early 1990s, US Renal Data System investigators showed a 27% increase in the relative risk of death associated with a serum phosphorus >6.5 mg/dl (4). Using the same data source, serum phosphorus >6.5 mg/dl was found to be significantly associated with sudden death and death due to coronary artery disease (5). More recently, we reported that serum phosphorus concentrations ≥5.0 mg/dl were associated with a significantly increased risk of death, hospitalization for cardiovascular disease, and fracture (6). These analyses were adjusted for case mix and a broad array of laboratory, including nutritional, variables. Finally, higher serum phosphorus has been associated with the extent of vascular calcification using B-mode ultrasound (7) and electron beam tomography (8,9).

The association between higher serum phosphorus and mortality has been further supported by experimental evidence. Jono and colleagues (2) demonstrated that the addition of phosphorus to cultured VSMC led to enhanced mineralization and

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

Address correspondence to: Dr. Glenn M. Chertow, University of California San Francisco, Department of Medicine Research, UCSF Laurel Heights, Suite 430, 3333 California Street, San Francisco, CA 94118-1211. Phone: 415-476-2173; Fax: 415-476-9531; Email: chertowg@medicine.ucsf.edu

ISSN: 1046-6673/1602-0293

expression of the osteoblast transcription factor core binding factor α-1 (Cbfa1). This transcription factor is felt to be critical for osteoblast differentiation from mesenchymal stem cells (10). Examination of the inferior epigastric arteries from kidney transplant recipients demonstrated enhanced expression of Cbfa1 in areas of calcification in the intima and medial layers (11). Subsequent work has demonstrated that the mineralizing effect of phosphorus on VSMC *in vitro* is augmented in the presence of higher calcium concentrations (12,13). Phosphorus appears to induce an osteoblast-like phenotype in VSMC. Once osteoblast-like genes and protein products are expressed, the VSMC are capable of mineralizing in the presence of phosphorus and calcium. Although Kestenbaum and colleagues did not evaluate the VA cohort for vascular calcification, matrix deposition leading to thickened medial layers may precede vascular calcification. These effects can manifest clinically as vascular noncompliance; vascular stiffness (as assessed by pulse wave analysis) and its proxy (pulse pressure) have both been associated with mortality in hemodialysis patients (7,14).

The authors note that the magnitude of the association between serum phosphorus and mortality in veterans with CKD was greater than that reported in hemodialysis patients by Block *et al.* (4) and Stevens *et al.* (15). The authors offered the following explanations for this observation: (*1*) inclusion of multiple phosphate measurements during the baseline period (thereby reducing misclassification), (*2*) differing ranges of phosphate levels among CKD *versus* ESRD populations, and (*3*) differing effects of phosphate among different patient populations. In the interest of considering biologic plausibility, we address each possibility.

First, while inclusion of multiple phosphate measurements could reduce misclassification, the authors report the median number of phosphorus determinations at 2, with an interquartile range of 1 to 4. In other words, fewer than half of the population had annual or more frequent determinations of serum phosphorus. As such, we would expect considerable misclassification of the serum phosphorus concentration due to biologic and random variation not captured by infrequent laboratory testing. Second, the range of serum phosphorus is much wider in ESRD than in CKD. Only 7% of veterans with CKD were hyperphosphatemic (≥4.5 mg/dl); approximately the same fraction of ESRD patients had serum phosphorus concentrations ≥8.5 mg/dl (6). Ironically, the relative risks of mortality associated with the "upper 7%" in the VA CKD cohort and a Fresenius Medical Care hemodialysis patient cohort (relative to below-the-median referent groups) were both approximately 1.9. We simply cannot generate a biologically plausible hypothesis for why the effects on mortality of a relatively small increase in serum phosphorus in CKD would be as extensive as those associated with much larger increases in serum phosphorus in ESRD. Rather, we would expect other ESRD-related factors (*e.g.*, uremia, hypercalcemia, more extensive inflammation, and secondary hyperparathyroidism, the provision of calcium-based phosphate binders and pharmacologic doses of vitamin D sterols, among other factors) to enhance the risk of vascular calcification in the hyperphosphatemic ESRD population. Third, we see no reason why the effects of serum phosphorus would be more extensive in veterans *versus* nonveterans; we previously reported no age-, sex-, or race-related differences in the relative risks of death and cardiovascular disease associated with hyperphosphatemia in ESRD.

Alternative Explanation

We propose that confounding by the severity of CKD is a more plausible explanation for the mortality risk profile described, rather than an augmentation of vascular calcification. First, any indirect measure of kidney function (*e.g.*, iothalamate or creatinine clearance, SCr or cystatin C) may over- or underestimate actual kidney function depending on test conditions and other confounding factors (*e.g.*, creatinine generation). Add to the potential "noise" of indirect measures of kidney function the regression coefficients incorporated into commonly applied GFR prediction equations, such as Cockcroft-Gault (16) or MDRD (17). Further add biologic changes that might develop over the course of a 3-yr baseline evaluation period.

Now imagine 1000 veterans with CKD, all with the same "time-weighted" SCr or eCrCl. How might we further gauge the severity of CKD? Arguably, the 500 less muscular veterans with lower creatinine generation rates would have more severe CKD (on average) than the 500 more muscular veterans with higher creatinine generation. The 500 veterans with lower hemoglobin concentrations would have more severe CKD (on average) than the 500 veterans with higher hemoglobin concentrations (assuming that erythropoietin had not been administered). The 500 veterans with lower serum bicarbonate concentrations would have more severe CKD (on average) than the 500 veterans with higher serum bicarbonate concentrations (assuming that base supplementation had not been administered). Similarly, the 500 veterans with higher serum phosphorus concentrations (after accounting for differences in body composition, hemoglobin, and bicarbonate) would have more severe CKD (on average) than the 500 veterans with lower serum phosphorus concentrations.

We should learn to expect misclassification of CKD severity when estimating kidney function using SCr or creatinine-based regression equations. As a result, we may bias risk estimates associated with CKD or stages of CKD in epidemiologic studies. Frequent proximate determinations of SCr or eGFR and the application of kidney function as a time-dependent covariate could attenuate misclassification (18). This more rigorous approach may not always be practical, particularly when evaluating CKD stages 2 and 3, where laboratory testing tends to be irregular and infrequent.

The Bottom Line

Our assertion that the marked increase in mortality risk associated with higher serum phosphorus in veterans with CKD reflects confounding by the severity of CKD (rather than a direct toxic effect of phosphorus) does not invalidate the results, just their interpretation. The associations are as described, and persons with CKD and higher serum phosphorus concentrations should, for the meantime, be considered to be at higher risk of mortality than other persons with CKD. Confirmation of these associations in other cohorts, particularly those with an ample proportion of women and persons of color, is warranted.

The overall mortality rate for veterans with CKD and at least one determination of serum phosphorus was quite high (>14% annually), confirming the distressingly high mortality rates

reported in other CKD cohorts (18). While the manuscript from Kestenbaum *et al.* extends the base of evidence that higher serum phosphorus concentrations are associated with an increased risk of mortality and cardiovascular disease in ESRD and in CKD, more work is urgently needed to understand the mechanism(s) involved. Because hyperphosphatemia and hypocalcemia are rare in mild to moderate CKD (19,20), more subtle abnormalities in mineral metabolism should be carefully investigated. As recommended by the Kidney Disease Quality Outcomes Initiative (K/DOQI), calcium, phosphorus and PTH should be measured regularly with CKD stages 3 and above. A 25-hydroxyvitamin D concentration should also be measured to evaluate for substrate deficiency, a common finding particularly among the elderly. Clinical trials comparing alternative management strategies for secondary hyperparathyroidism in CKD (*e.g.*, dietary phosphate restriction *versus* phosphate binders *versus* activated vitamin D analogues *versus* other agents) would require creative determination of outcomes and extended follow-up times, but would prove extremely valuable.

Finally, these findings should encourage investigators interested in refining classification and stratification of CKD to look beyond GFR, toward parameters of tubular function and other overt manifestations of lower GFR that might help reduce misclassification of SCr-based GFR estimates (*e.g.*, hemoglobin, bicarbonate, and phosphorus, among others). While reducing calcification should be a priority in any treatment strategy for hyperphosphatemia and secondary hyperparathyroidism, enhancing CKD classification should also improve care and outcomes for patients with CKD.

References

1. Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, Sherrard DJ, Andress DL: Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol* 16: 520–528, 2005
2. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM: Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 87: E10–E17, 2000
3. Kausz AT, Khan SS, Abichandani R, Kazmi WH, Obrador GT, Ruthazer R, Pereira BJ: Management of patients with chronic renal insufficiency in the Northeastern United States. *J Am Soc Nephrol* 12: 1501–1507, 2001
4. Block GA, Hulbert-Shearon TE, Levin NW, Port FK: Association of serum phosphorus and calcium × phosphate product with mortality risk in chronic hemodialysis patients: A national study. *Am J Kidney Dis* 31: 601–617, 1998
5. Ganesh SK, Stack AG, Levin NW, Hulbert-Shearon T, Port FK: Association of elevated serum PO_4, Ca × PO_4 product, and parathyroid hormone with cardiac mortality risk in chronic hemodialysis patients. *J Am Soc Nephrol* 12: 2131–2138, 2001
6. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM: Mineral metabolism, mortality and morbidity in maintenance hemodialysis. *J Am Soc Nephrol* 15: 2208–2218, 2004
7. Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM: Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension* 38: 938–942, 2001
8. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, Salusky IB: Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 342: 1478–1483, 2000
9. Raggi P, Boulay A, Chasan-Taber S, Amin N, Dillon M, Burke SK, Chertow GM: Cardiac calcification in adult hemodialysis patients: A link between ESRD and cardiovascular disease? *J Am Coll Cardiol* 39: 695–701, 2002
10. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G: Osf2/Cbfa1: A transcriptional activator of osteoblast differentiation. *Cell* 89: 747–754, 1997
11. Moe SM, Duan D, Doehle BP, O'Neill KD, Chen NX: Uremia induces the osteoblast differentiation factor Cbfa1 in human blood vessels. *Kidney Int* 63: 1003–1011, 2003
12. Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, Jahnen-Dechent W, Weissberg PL, Shanahan CM: Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: A potential mechanism for accelerated vascular calcification in ESRD. *J Am Soc Nephrol* 15: 2857–2867, 2004
13. Yang H, Curinga G, Giachelli CM: Elevated extracellular calcium levels induce smooth muscle cell matrix mineralization in vitro. *Kidney Int* 66: 2293–2299, 2004
14. Klassen PS, Lowrie EG, Reddan DN, DeLong ER, Coladonato JA, Szczech LA, Lazarus JM, Owen WF Jr: Association between pulse pressure and mortality in patients undergoing maintenance hemodialysis. *JAMA* 287: 1548–1555, 2002
15. Stevens LA, Djurdjev O, Cardew S, Cameron EC, Levin A: Calcium, phosphate, and parathyroid hormone levels in combination and as a function of dialysis duration predict mortality: Evidence for the complexity of the association between mineral metabolism and outcomes. *J Am Soc Nephrol* 15: 770–779, 2004
16. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16: 31–41, 1976
17. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 130: 461–470, 1999
18. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY: Chronic kidney disease and risk of death, cardiovascular events and hospitalization. *N Engl J Med* 351: 1296–1305, 2004
19. Martinez I, Saracho R, Montenegro J, Llach F: The importance of dietary calcium and phosphorus in the secondary hyperparathyroidism of patients with early renal failure. *Am J Kidney Dis* 29: 496–502, 1997
20. Hsu CY, Chertow GM: Elevations of serum phosphorus and potassium in mild to moderate chronic renal insufficiency. *Nephrol Dial Transplant* 17: 1419–1425, 2002

See related editorial, "Serum Phosphate Levels and Mortality Risk among People with Chronic Kidney Disease," on pages 520–528.