Changes in serum calcium, phosphate, and PTH and the risk of death in incident dialysis patients: A longitudinal study

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Elevated bone mineral parameters have been associated with mortality in dialysis patients. There are conflicting data about calcium, parathyroid hormone (PTH), and mortality and few data about changes in bone mineral parameters over time. We conducted a prospective cohort study of 1007 incident hemodialysis and peritoneal dialysis patients. We examined longitudinal changes in bone mineral parameters and whether their associations with mortality were independent of time on dialysis, inflammation, and comorbidity. Serum calcium, phosphate, and calcium–phosphate product (CaP) increased in these patients between baseline and 6 months ($P<0.001$) and then remained stable. Serum PTH decreased over the first year ($P<0.001$). In Cox proportional hazards models adjusting for inflammation, comorbidity, and other confounders, the highest quartile of phosphate was associated with a hazard ratio (HR) of 1.57 (1.07–2.30) using both baseline and time-dependent values. The highest quartiles of calcium, CaP, and PTH were associated with mortality in time-dependent models but not in those using baseline values. The lowest quartile of PTH was associated with an HR of 0.65 (0.44–0.98) in the time-dependent model with 6-month lag analysis. We conclude that high levels of phosphate both at baseline and over follow-up are associated with mortality in incident dialysis patients. High levels of calcium, CaP, and PTH are associated with mortality immediately preceding an event. Promising new interventions need to be rigorously tested in clinical trials for their ability to achieve normalization of bone mineral parameters and reduce deaths of dialysis patients.

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Patients with end-stage renal disease are at higher risk for mortality than the general population.[1] The primary cause of death in end-stage renal disease is cardiovascular disease (CVD). Patients with end-stage renal disease have many of the same risk factors for mortality as the general population, but the higher risk seen in dialysis patients cannot be fully explained by prevalence of traditional risk factors.[2] Novel risk factors for mortality, including anemia and abnormal calcium and phosphate metabolism,[3] have been proposed to explain this greater risk of CVD.

High levels of serum calcium, phosphate, calcium–phosphate product (CaP), and parathyroid hormone (PTH) have been shown to be associated with all-cause and cardiovascular-specific mortality in persons with kidney disease.[4–8] In contrast, other studies have shown that low PTH and calcium levels are associated with mortality[9,10] or no association at all.[11] These conflicting results may be due to use of secondary databases, prevalent patients, and single measures of calcium, phosphate, and PTH. Importantly, there have been few data on prospective changes in bone mineral metabolic parameters; therefore, use of a single value has not been validated. If prospective changes exist in bone mineral parameters, this may explain some of the previous conflicting results using a single measure in patients with different dialysis durations.

Despite limitations in prior epidemiologic studies, several therapeutic agents have been developed and are increasingly used based on the assumption that the described relationships are causal in nature and that optimization of bone mineral metabolic parameters will lead to improvement in outcome and patient survival. No clinical trial to date proves this hypothesis. We therefore tested the hypothesis that abnormal bone mineral parameters are associated with mortality in a large prospectively collected national cohort of incident dialysis patients with repeated measures of laboratory values, detailed information on a variety of

important confounders, and validated outcomes in order to quantitate these relationships more precisely and to examine whether associations are truly independent of potential confounders, such as inflammation. Such information is essential to facilitate the appropriate study and use of the new interventions that target bone mineral parameters.

RESULTS

Patient characteristics

Our sample included 746 hemodialysis patients and 259 peritoneal dialysis patients from the CHOICE (Choices for Healthy Outcomes in Caring for End-Stage Renal Disease (ESRD)) (Table 1). Patients were followed for a median of 2.5 (intraquartile range 1.3–4.3) years with 460 deaths occurring during follow-up. Patients in the highest calcium quartile were more likely to be female, have a hemoglobin >10.8 g/dl, be non-African American and were less likely to have PTH >160 pg/ml relative to patients in lower quartiles. Patients in the highest quartile of serum phosphate compared to the other quartiles were less likely to be >65 years old, African American, or to have been referred to a nephrologist <4 months prior to initiating dialysis but were more likely to have smoked. The highest quartile of PTH had a greater percentage of patients who were African American, high school graduates, employed, had a body mass index (BMI) >25 kg/m^2, had an albumin >3.7 g/dl, and were on injectable vitamin D therapy at baseline, but had fewer patients with a hemoglobin >10.8 g/dl, with diabetes mellitus and who were referred to a nephrologist <4 months prior to initiation of dialysis. Phosphate explained 91% of the variance of CaP at baseline. Trends in CaP mirrored those of phosphate alone (data not shown), except that patients with higher BMIs tended to have higher CaPs and late referral to a nephrologist was not significantly associated with CaP.

Longitudinal changes in bone mineral parameters

Mean serum calcium, phosphate, and CaP significantly increased between the baseline period and the 6-month period: calcium, from 9.35 to 9.51 mg/dl ($P<0.001$); phosphate, from 5.23 to 5.43 mg/dl ($P<0.001$); and CaP, from 48.9 to 51.7 mg^2/dl^2 ($P<0.001$ for all). After 6 months of follow-up, mean serum calcium, phosphate, and CaP remained stable (Figure 1). Serum PTH levels fell after the initiation of dialysis and continued to fall for the next year. Median serum PTH then increased reaching a new plateau at around 2 years (Figure 1). Using generalized estimating equations modeling to assess the association of bone mineral parameters with increasing Index of Coexistent Disease (ICED) score, we found that per 1-unit increase in ICED score, calcium increased 0.07 mg/dl (0.03–0.10) ($P=0.001$), phosphate increased 0.09 mg/dl (0.01–0.17) ($P=0.03$), CaP increased 1.10 mg^2/dl^2 (0.31–1.88) ($P=0.006$), and PTH decreased by 14.6 pg/ml (-34.4 to 5.16) ($P=0.15$).

Association between serum calcium and all-cause mortality

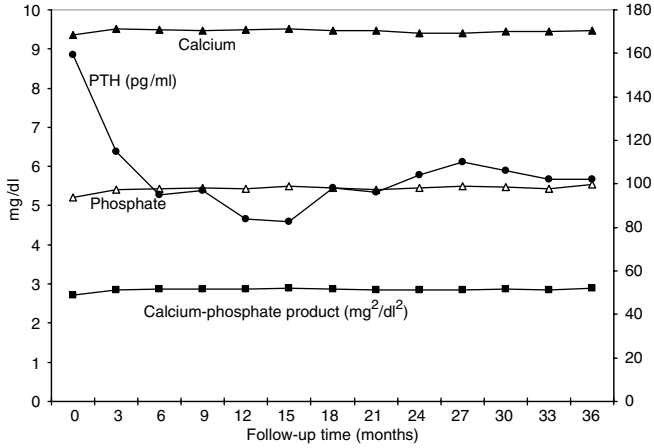
Table 2 shows the association between serum calcium quartiles and mortality adjusted for potential confounders, using baseline, time-dependent, and time-dependent with lag models. The highest quartile of serum calcium was significantly associated with mortality in time-dependent

Table 1 | Patient characteristics at baseline, stratified by serum calcium, phosphate, and PTH quartiles

		Serum calcium quartile (mg/dl)				Serum phosphate quartile (mg/dl)				Serum PTH quartile (pg/ml)			
Characteristic (*n*)	All patients (1007) (%)	<8.97 (251) (%)	8.97–9.33 (254) (%)	9.33–9.73 (250) (%)	>9.73 (252) (%)	<4.3 (248) (%)	4.3–5.1 (260) (%)	5.1–6 (243) %	>6 (259) %	<76 (166) %	76–160 (167) (%)	160–308 (165) (%)	>308 (168) (%)
Age >65 years	36	37	35	38	35	**47**	**44**	**32**	**24**	38	38	40	30
Female	46	**38**	**43**	**44**	**59**	50	48	44	43	51	41	40	49
African American	28	**24**	**32**	**32**	**21**	**31**	**31**	**26**	**23**	**18**	**17**	**33**	**41**
Hemodialysis	74	**68**	**76**	**79**	**74**	**67**	**73**	**80**	**77**	83	84	81	83
Ever smoked	61	61	64	58	60	**54**	**61**	**60**	**67**	62	58	65	53
Baseline CVD	57	56	63	59	52	60	59	58	53	60	62	58	54
Diabetes mellitus	55	55	60	55	49	53	52	56	58	**56**	**62**	**63**	**45**
ICED 2	34	33	31	35	39	34	32	31	41	37	37	32	32
ICED 3	30	27	32	33	28	31	32	34	23	36	28	32	29
Late referral <4 months (805)	30	26	30	34	30	**32**	**36**	**30**	**22**	**38**	**31**	**27**	**19**
High school grade (944)	70	68	72	68	73	66	70	70	74	**68**	**79**	**64**	**74**
Employed	13	**17**	**13**	**8**	**14**	10	14	12	17	**10**	**11**	**12**	**20**
BMI >25 kg/m^2	59	62	58	59	55	53	56	64	61	**51**	**52**	**67**	**67**
Calcitriol use	40	44	44	38	34	42	41	39	39	**31**	**41**	**50**	**55**
CRP >0.4 (844)	48	47	46	48	49	50	50	46	45	51	45	45	45
IL-6 >6.0 (844)	51	47	53	52	50	52	54	49	47	52	47	51	47
Alb >3.7	48	46	47	49	48	45	47	44	55	**39**	**49**	**49**	**55**
Hgb >10.8	51	**43**	**52**	**48**	**60**	53	55	47	48	**52**	**56**	**49**	**40**
PTH >160 (666)	50	**75**	**57**	**46**	**23**	43	49	52	56				

Alb, albumin (g/dl); BMI: body mass index; CRP, C-reactive protein (mg/dl); CVD, cardiovascular disease; Hgb, hemoglobin (g/dl); ICED, Index of Coexistent Disease; IL-6, interleukin-6 (pg/ml); PTH, parathyroid hormone (pg/ml),
Boldface values represent $P<0.05$.

analysis, but not when using baseline and time-dependent with lag analyses. The time-dependent model showed that having a calcium >9.73 mg/dl was associated with a 52% increased risk of death, compared to the reference range (8.97–9.33 mg/dl).



CaP *n*	1007	978	912	834	776	718	667	581	510	434	355	267	210
PTH *n*	666	467	455	433	428	392	383	307	293	247	200	147	120

Figure 1 | Mean serum calcium, phosphate, CaP product, and median serum PTH values over the first 36 months after initiation of dialysis.

Association between serum phosphate and all-cause mortality

The relationship between serum phosphate and mortality is shown in Table 2. The highest quartile of phosphate (>6.0 mg/dl) was associated with a higher mortality in the baseline and time-dependent models, with a 57% higher risk of death for the highest quartile compared with the reference quartile (4.3–5.1 mg/dl) in the time-dependent model. In the time-dependent model with lag, the third phosphate quartile (5.1–6.0 mg/dl) was associated with an 83% higher mortality risk than the reference quartile.

Association between serum calcium–phosphate product and all-cause mortality

In the baseline and time-dependent with lag models, there was no association between CaP and mortality (Table 2). However, using time-dependent CaP values, the highest quartile of CaP (>56.4 mg^2/dl^2) was associated with an 87% higher risk of mortality compared to the reference quartile (40.2–47.7 mg^2/dl^2).

Association between parathyroid hormone and all-cause mortality

There was no association between baseline PTH values and mortality (Table 2). In the time-dependent with lag analysis,

Table 2 | Associations of serum calcium, phosphate, CaP, and PTH at different time points with all-cause mortality

	Baseline values as predictors (*n*=552)	Time-dependent predictors with 6-month lag (*n*=578)	Time-dependent predictors (*n*=593)
Serum calcium (mg/dl) quartile: level			
<8.97	0.92 (0.60–1.39)	0.91 (0.58–1.43)	1.31 (0.83–2.05)
8.97–9.33	1.0 (reference)	1.0 (reference)	1.0 (reference)
9.33–9.73	1.13 (0.78–1.64)	1.17 (0.76–1.80)	1.33 (0.85–2.07)
>9.73	1.05 (0.69–1.62)	1.37 (0.93–2.02)	1.52 (1.02–2.26)*
Serum phosphate (mg/dl) quartile: level			
<4.3	1.04 (0.70–1.53)	1.36 (0.89–2.06)	1.10 (0.74–1.64)
4.3–5.1	1.0 (reference)	1.0 (reference)	1.0 (reference)
5.1–6.0	1.01 (0.69–1.47)	1.83 (1.20–2.79)**	1.08 (0.70–1.67)
>6.0	1.54 (1.01–2.35)*	1.41 (0.93–2.13)	1.57 (1.07–2.30)*
Serum CaP (mg^2/dl^2) quartile: level			
<40.2	0.94 (0.64–1.39)	1.11 (0.73–1.68)	1.30 (0.85–1.98)
40.2–47.7	1.0 (reference)	1.0 (reference)	1.0 (reference)
47.7–56.4	1.03 (0.71–1.49)	1.37 (0.90–2.07)	1.50 (0.98–2.31)
>56.4	1.35 (0.89–2.05)	1.28 (0.86–1.89)	1.87 (1.25–2.80)**
Serum PTH (pg/ml) quartile: level			
<76	0.77 (0.51–1.19)	0.65 (0.44–0.98)*	0.86 (0.55–1.33)
76–160	1.03 (0.68–1.55)	0.67 (0.43–1.04)	1.01 (0.64–1.59)
160–308	1.0 (reference)	1.0 (reference)	1.0 (reference)
>308	0.84 (0.55–1.28)	1.23 (0.76–1.98)	1.68 (1.01–2.78)*
No vitamin D medication use	1.0 (reference)	1.0 (reference)	1.0 (reference)
Vitamin D medication use	0.62 (0.44–0.86)*	0.73 (0.54–0.98)*	0.74 (0.56–1.00)*

CaP, calcium–phosphate product; PTH, parathyroid hormone.
*$P<0.05$; **$P<0.01$.
Models adjusted for age, sex, race, baseline modality, smoking (ever vs. never), body mass index, Index of Coexistent Disease category, baseline diabetes mellitus, baseline cardiovascular disease, log baseline interleukin-6, log baseline C-reactive protein, time-dependent albumin, time-dependent hemoglobin, education, employment status, late referral, and vitamin D medication use (only in non-baseline models). Calcium, phosphate, and CaP models adjusted for PTH. PTH model adjusted for calcium and phosphate. Vitamin D medication use adjusted for calcium, phosphate, and PTH.

there was no association between an elevated PTH and mortality; however, having a PTH < 76 pg/ml was associated with approximately a 35% lower risk of mortality. In the time-dependent analysis, the highest quartile of PTH (> 308 pg/ml) was associated with a 68% greater risk of mortality compared to the reference quartile (160–308 pg/ml).

Association between injectable calcitriol and all-cause mortality

Patients who received injectable vitamin D in the population had a 26% lower mortality compared to those who did not receive vitamin D (Table 2); this association of injectable vitamin D treatment with reduced mortality was seen in all statistical models.

Six-month changes in calcium, phosphate, and calcium–phosphate product and all-cause mortality

Patients who had increasing levels of calcium had a 76% higher mortality risk than those with low/normal levels at both time points (Table 3). Having serum phosphate levels decrease from baseline to 6 months was associated with a 72% higher mortality compared to those who had low/normal levels at both time points. In a sensitivity analysis excluding patients with a serum phosphate < 3.5 mg/dl, having serum phosphate levels decrease from baseline to 6 months was associated with a hazard ratio of 1.82 (1.12–2.98).

Subgroup analysis

Subgroup analyses using time-dependent models revealed no differences in the above relationships between hemodialysis and peritoneal dialysis patients or patients with and without diabetes mellitus (Table 4). Although the p-interactions were not significant between different dialysis modalities, the peritoneal dialysis subgroup was too small to allow for valid multivariate analysis. In the time-dependent models, adding non-adherence or Kt/V as adjusters did not change the association between elevated serum phosphate and mortality; the highest quartile of phosphate (> 6.0 mg/dl) had a hazard ratio of 1.67 (1.08–2.59) adjusting for non-adherence, and 1.63 (1.01–2.62) adjusting for Kt/V.

DISCUSSION

There have been no clinical trials showing that normalization of bone mineral parameters decreases mortality; the next best study to evaluate these associations is a prospective cohort study, such as the CHOICE Study, which is able to take into account changes in bone mineral parameters over time. The results from this nationally representative dialysis cohort study show that levels of mean serum calcium, phosphate, and CaP increased from baseline to 6 months and then remained stable. Serum calcium is low in patients with chronic kidney disease, and upon initiating dialysis, patients commonly receive vitamin D replacement therapy, calcium-based phosphate binders, and calcium in dialysate fluid. Therefore, serum calcium levels would be expected to increase after initiation of dialysis. However, we found that serum phosphate levels increased during this time period as well and that increasing levels of comorbidity were associated with increasing levels of calcium and phosphate. Block *et al.*,[5] in their analysis of a prevalent population, also found that higher levels of phosphate were found in patients who had been on dialysis for a longer time period. Serum phosphate levels rebound after dialysis treatment,[12] and vitamin D medications, such as calcitriol, can increase patients' serum phosphate by increasing its intestinal absorption. Another factor contributing to the increasing phosphate level over time could be loss of residual renal function.

The relationship between serum calcium and mortality remains unclear. In the Dialysis Morbidity and Mortality Study Wave 1, Block *et al.*[4] showed no relationship between levels of serum calcium and mortality after adjustment for serum albumin. Foley *et al.*[10] reported an increased mortality in patients with a low serum calcium level (< 8.8 mg/dl). Block *et al.*,[5] in a larger study, suggested that there is a graded association between higher serum calcium and higher mortality risk. In this analysis, high levels of serum calcium were associated with mortality only in time-dependent models, but not in baseline or lagged time-dependent models. Also, as mentioned above, we saw that increasing calcium levels were associated with increasing comorbidity.

In this study, we were able to show that serum phosphate had an association with mortality that was independent of inflammation, comorbidity, and non-adherence and robust

Table 3 | Relative risk of all-cause mortality in patients surviving 6 months by change in the level of serum calcium, serum phosphate, and CaP

	Serum calcium 9.73 mg/dl		Serum phosphate 6.0 mg/dl		Serum CaP 56 mg^2/dl^2	
Levels at baseline and 6 months	*N*	Relative risk (95% CI)	*N*	Relative risk (95% CI)	*N*	Relative risk (95% CI)
Low/normal	498	1.0 (reference)	538	1.0 (reference)	509	1.0 (reference)
Increase	185	1.76** (1.23–2.51)	148	0.92 (0.63–1.35)	154	1.14 (0.78–1.67)
Decrease	85	1.32 (0.83–2.09)	103	1.72* (1.06–2.79)	90	1.60 (0.97–2.62)
High	133	1.19 (0.74–1.90)	118	1.36 (0.84–2.21)	146	1.33 (0.86–2.04)

CaP, calcium–phosphate product; CI, confidence interval.[2] *$P < 0.05$; **$P < 0.01$.
All models adjusted for age, sex, race, baseline modality, smoking (ever vs never), body mass index, Index of Coexistent Disease category, diabetes mellitus, baseline cardiovascular disease, log interleukin-6, log C-reactive protein, time-dependent albumin and hemoglobin and education, vitamin D medication status, employment status, and late referral. Calcium, phosphate, and CaP models adjusted for parathyroid hormone.

Table 4 | Baseline values and time-dependent associations with mortality in hemodialysis and peritoneal dialysis subgroups and in patients with and without diabetes

	Hemodialysis (482)	Peritoneal dialysis (111)	*P*- value*	Diabetes mellitus (329)	No diabetes mellitus (264)	*P*- value*
Mean values at baseline by subgroup						
Calcium	9.39 (0.63)	9.31 (0.73)	0.09	9.34 (0.65)	9.40 (0.67)	0.17
Phosphate	5.28 (1.30)	5.04 (1.38)	0.01	5.25 (1.32)	5.19 (1.34)	0.50
CaP	49.6 (12.6)	46.8 (12.3)	0.002	48.9 (12.2)	48.8 (13.0)	0.89
PTH	158 (77–311)	164 (74–299)	0.60	151 (77–267)	176 (74–391)	0.06
Time-dependent associations with mortality in the highest quartile						
Calcium	1.60 (1.03–2.49)	—	0.83	1.65 (0.95–2.86)	1.16 (0.56–2.42)	0.66
Phosphate	1.71 (1.11–2.64)	—	0.32	2.14 (1.25–3.67)	0.84 (0.38–1.86)	0.10
CaP	2.08 (1.32–3.29)	—	0.39	2.76 (1.58–4.83)	1.13 (0.48–2.64)	0.21
PTH	1.79 (1.01–3.17)	—	0.78	1.85 (0.91–3.78)	2.04 (0.73–5.71)	0.42

CaP, calcium–phosphate product; PTH, parathyroid hormone.
Reported as means (s.d.) or median (intraquartile range). **P*-values reported from *t*-tests and Wilcoxon rank-sum tests for comparisons between groups and from interaction terms in time-dependent models, adjusted as in Table 2.
—, numbers too small to give valid estimate.

in several different statistical models. While there have been no clinical trials evaluating decreasing phosphate levels and mortality, The Treat to Goal study showed less progression in aortic calcification in patients treated with sevelamer compared to calcium-based binders but did not evaluate differences in mortality.[13] In a secondary analysis, we found that patients whose phosphate levels were high at baseline but subsequently decreased significantly by 6 months had a higher mortality relative to those whose levels were low at both time points. Eliminating those with very low serum phosphate (<3.5 mg/dl) did not change this finding. The underlying mechanism is not clear but may involve poor oral intake and/or the aggressive use of calcium-based phosphate binders, which may both decrease the phosphate and increase the calcium, both of which were found to be associated with increased mortality in our analysis. We did not have information on oral medications and therefore could not account for their use in our analysis. Further studies are needed to clarify these associations.

Laboratory and clinical studies have revealed possible biological mechanisms to explain the relationship between bone mineral parameters and mortality. High serum phosphate level can differentiate vascular smooth muscle cells into cells with osteoblastic activity, thereby promoting mineralization of the vascular system.[14,15] Higher calcium intake is also associated with increased coronary calcification in young hemodialysis patients.[16] Raggi *et al.*[17] similarly showed an association between higher serum calcium and phosphate levels and coronary calcification in a larger population, as well as cross-sectional association of calcifications with myocardial infarction, angina, and claudication. Calcification has been associated with increased arterial stiffness and mortality.[18,19]

PTH can induce cardiomyocyte hypertrophy *in vitro*, and, in a population-based study in Norway, epidemiological evidence linked higher levels of PTH to left ventricular hypertrophy.[20,21] We found that the highest quartile of PTH in the time-dependent analysis was associated with a 70% elevated mortality risk compared to a level of 160–308 pg/ml, one of the highest risk estimates thus far reported.[6,9] However, using baseline and lagged analyses, we did not find an association between high PTH and mortality. We found that PTH levels <76 pg/ml were associated with a lower mortality risk compared to levels of 160–310 pg/ml in a lagged analysis. These findings are not entirely consistent with previous data regarding associations of altered PTH levels and mortality or vascular calcification.[9,22,23] Similar to our findings, Kestenbaum *et al.*[24] reported that post-parathyroidectomy patients, presumably with lower PTH levels, have a survival benefit compared to matched controls. These inconsistencies may result from poor agreement of different PTH assays used in different studies and possibly from the smaller sample size used in this analysis.

In our analysis, patients who had received calcitriol had a survival advantage over patients who did not use calcitriol. This benefit was most pronounced in the baseline analysis most likely owing to a survival bias; patients had to have survived long enough on dialysis to have received calcitriol. This survival advantage of patients treated with calcitriol and other injectable vitamin D medications has been reported previously.[11]

There are limitations to this study. It is essential to emphasize that like all other studies of bone mineral parameters and mortality published to date, this is an observational study and therefore causality cannot be directly inferred. The sample size in our study is smaller than in other studies; however, we were able to obtain repeated measures of calcium, phosphate, CaP, and PTH. Over the time course of the study, clinical practice has changed, with the introduction of non-calcium-containing phosphate binders and new vitamin D analogs, which we and others have not accounted for in the analysis. Finally, although we have information on whether or not patients received calcitriol, we could not quantify the dosage.

In summary, elevated phosphate was independently associated with all-cause mortality in a nationally represen-

tative incident dialysis cohort, even after accounting for measures of inflammation, education, employment, referral time, and other confounders. Elevated calcium, CaP, and PTH were associated with mortality only in time-dependent models, suggesting that the association may be more complex than previously understood. Our analysis also shows that interventions used during the time of the study were inadequate in lowering phosphate levels. This and other observational studies suggest that clinicians need to target serum phosphate aggressively to prevent death in end-stage renal disease patients. Our analysis strengthens the potential impact of NKF-KDOQI guidelines for normalization of bone mineral parameters.[25] The evident complexity of the associations between bone mineral levels and survival mandates that potential new interventions and enhanced methods to implement them need to be rigorously tested in appropriately sized clinical trials for their ability to achieve normalization of bone mineral parameters and reduce deaths of dialysis patients.

MATERIALS AND METHODS

Study design and research population

We conducted a national cohort study using a subcohort from the CHOICE Study. CHOICE is a national, prospective cohort study initiated to investigate treatment choices and outcomes of dialysis care among patients initiating dialysis. From October 1995 to June 1998, 1041 (767 hemodialysis and 274 peritoneal dialysis) patients were enrolled from 81 dialysis clinics in 19 states; enrollment occurred a median of 45 days after the start of dialysis (98% within 4 months). These included clinics associated with Dialysis Clinic Inc. (Nashville, TN, USA; $n = 923$), New Haven CAPD (New Haven, CT, USA; $n = 86$), and St Raphael's Hospital (New Haven, CT, USA; $n = 32$). Patients enrolled in CHOICE were similar to patients initiating dialysis in the United States in 1997 except that CHOICE oversampled peritoneal dialysis patients to allow for comparisons.[2,26] Entry criteria included the initiation of chronic outpatient dialysis in the preceding 3 months, ability to provide informed consent for participation, age older than 17 years, and ability to speak English or Spanish. A specimen bank was established to store blood samples from the Dialysis Clinic Inc. participants, and specimens were obtained for 898 (97.3%) of the Dialysis Clinic Inc. enrollees. Patients included in this analysis had to have serum calcium and phosphate at baseline ($n = 1007$). The Johns Hopkins University School of Medicine Institutional Review Board and the review boards for the clinical centers approved the study protocol. All patients gave written informed consent before participation in the study.

Data collection

Dialysis modality at baseline was defined as the modality at 4 weeks after enrollment in the study and was categorized as hemodialysis or peritoneal dialysis. Data regarding patient demographics and medical history were collected from a self-report questionnaire and chart review. Baseline CVD was coded as positive if the patient had a history of coronary artery disease, myocardial infarction, cerebrovascular disease, peripheral vascular disease, or transient ischemic attack at dialysis initiation. Patients were categorized as non-adherent (yes/no) if they had missed $>3\%$ of their hemodialysis sessions per month, after accounting for missed dialysis secondary to hospitalization.[27] No adherence data were available for peritoneal dialysis patients. The degree of comorbidity was assessed using the ICED, an instrument that has been validated in dialysis populations.[28,29] The ICED score ranges from 0 to 3 (with 3 indicating highest severity), and is calculated from two constituent indices that were assessed at baseline and 12 months, the 19-axis Index of Disease Severity and the 11-axis Index of Physical Impairment.

Laboratory data included repeated measures of serum calcium, phosphate, PTH, albumin, and hemoglobin. The baseline laboratory parameters were those collected in the 3 months surrounding enrollment in the study (45 days before and 45 days after enrollment). PTH levels were measured using the Diasorin intact PTH assay (Diasorin Inc., Stillwater, MN, USA). Serum calcium levels reported in the study were adjusted for albumin using the following formula: adjusted calcium = measured calcium $-((4.0 -$ serum albumin in g/dl$) \times 0.8)$. High-sensitivity C-reactive protein (CRP) and interleukin-6 (IL-6) were performed on all patients with frozen serum available in the CHOICE specimen bank ($n = 844$). Single-pool K_t/V at 6 months, calculated using previously published methods,[30] was available for hemodialysis patients. Injectable vitamin D use data, available for calcitriol only, were obtained from linked United States Renal Data System billing data. Patients were categorized as having received or not received injectable vitamin D (calcitriol) during follow-up. Mortality was ascertained through communication with the dialysis clinics and monthly reports from the central Dialysis Clinic Inc. database.

Statistical analysis

Characteristics of the population stratified by serum calcium, phosphate, PTH, and CaP quartiles were compared using χ^2 tests for categorical variables. CRP, IL-6, albumin, hemoglobin, and PTH levels were categorized based on the median baseline value of the population. Mean serum calcium, phosphate, and CaP levels at baseline and 6 months were compared using paired Student's t-tests. PTH values were compared using the Wilcoxon rank-sum test. To assess whether increasing comorbidity (ICED) is associated with changes in bone mineral parameters, we used generalized estimating equations to estimate the changes in bone mineral parameters from baseline to 1 year associated with increased ICED from baseline to 1 year, adjusting for age, sex, and race.

We used Cox proportional hazards analysis to assess the presence, strength, independence, and statistical significance of the association of mean quarterly bone mineral levels with all-cause mortality. The mean serum calcium, phosphate, CaP, PTH, albumin, and hemoglobin levels using all available results for each quarter were used as the exposure for each 3-month follow-up period. When there were no available results for a given parameter within a specific quarter (13% in the first year, 16% in the second year for calcium and phosphate), values were assigned using the last observation carried forward method. To examine the influence that secular trends in bone mineral levels might have on mortality, we ran separate analyses using baseline values only (3 months surrounding enrollment), using updated values of bone mineral parameters in a time-dependent fashion, and using time-dependent values with a 6-month lag (i.e. allowing bone mineral parameter to change with time only up to 6 months before the event). Adjustment was performed using the potential confounders age, race, sex, diabetes status, baseline modality, baseline CVD, ICED, referral time to a nephrologist, education, employment, time-dependent albumin, time-dependent hemoglobin, CRP, IL-6, vitamin D medication use,

and the remaining bone mineral parameters: PTH for the calcium, phosphate, and CaP models and serum calcium and phosphate for the PTH models. For the baseline values models, vitamin D medication use was not included in the model because patients had to have survived to use vitamin D, thereby introducing a survival bias to the estimates. All analyses were stratified by dialysis clinic to account for intraclinic practice variation. Patients were censored at transplantation, transfer to another dialysis clinic, or last date of follow-up (November 30, 2004).

Values of mineral parameters were categorized according to quartiles of the baseline distribution. Quartile 2 was used as the reference category for analyses of serum calcium, phosphate, and CaP because of previous data showing a possible J-shaped association with mortality.[5,10] Quartile 3 was used as the reference category for PTH because the cutoffs approximated the goal PTH level under current guidelines.[25] Subgroup analyses were performed to evaluate whether there were differences in the association between bone mineral parameters and mortality between hemodialysis and peritoneal dialysis patients or between patients with and without baseline diabetes mellitus, and sensitivity analysis was performed to evaluate the separate effects of Kt/V and non-adherence.

To further examine individual changes in mineral parameters over time, we looked at minerals separately using baseline and 6-month patient-specific levels. A categorical variable was created defining four groups of patients: those persistently below the 75th percentile (low-normal; reference group), those persistently above the 75th percentile at both time points (high), those that changed from above to below the 75th percentile (decrease), and those that changed from below to above the 75th percentile (increase). This categorical variable was then used in the final model, as described above. In a sensitivity analysis, we excluded patients with a serum phosphate less than 3.5 mg/dl. Statistical analyses were performed using Stata software, version 8.1 (Stata Corporation, College Station, TX, USA).

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