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Survival predictability of time-varying indicators of bone disease in maintenance hemodialysis patients

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Although renal osteodystrophy and vitamin D analogs may be related to survival in maintenance hemodialysis (MHD) patients, most studies have examined associations between baseline values and survival without accounting for variations in clinical and laboratory measures over time. We examined associations between survival and quarterly laboratory values and administered paricalcitol in a 2-year (July 2001–June 2003) cohort of 58 058 MHD patients from all DaVita dialysis clinics in USA using both time-dependent Cox models with repeated measures and fixed-covariate Cox models with only baseline values. Whereas hypercalcemia and hyperphosphatemia were robust predictors of higher death risk in all models, the association between serum calcium and mortality was different in time-varying models. Changes in baseline calcium and phosphorus values beyond the Kidney Disease Outcome Quality Initiative recommended targets were associated with increased mortality. Associations between high serum parathyroid hormone and increased death risk were masked by case-mix characteristics of MHD patients. Time-varying serum alkaline phosphatase had an incremental association with mortality. Administration of any dose of paricalcitol was associated with improved survival in time-varying models. Controlling for nutritional markers may introduce overadjustment bias owing to their strong collinearity with osteodystrophy surrogates. Whereas both time-dependent and fixed-covariate Cox models result in similar associations between osteodystrophy indicators and survival, subtle but potentially clinically relevant differences between the two models exist, probably because fixed models do not account for variations of osteodystrophy indices and changes in medication dose over time.

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Renal osteodystrophy is a common complication of advanced chronic kidney disease (stages 3–5) and may be associated with disorders of mineral metabolism, coronary artery calcifications, and poor survival, especially among those who require maintenance dialysis treatment.[1–5] Several recent studies[6–9] including a large epidemiologic study by Block *et al.*[10] have shown an association between indices of the renal osteodystrophy management and mortality in maintenance hemodialysis (MHD) patients. However, virtually all these studies have examined associations between the *baseline* serum mineral values at the start of a given cohort and subsequent survival not accounting for the *changes* in concentrations of these measures and other covariates over time. Blood concentrations of minerals (calcium and phosphorus), parathyroid hormone (PTH), and alkaline phosphatase may change greatly over time. Such changes may be the result of variations in administered medications such as vitamin D analogs or the type of phosphorus binders or owing to longitudinal alterations in other biological factors such as alterations in factors related to malnutrition–inflammation–cachexia syndrome (MICS). Because the medical management of MHD patients by clinicians is usually based on *repeated* measurements of blood tests, examining such associations using time-dependent models that are not restricted to fixed baseline data but include changes over time is more similar to real clinical scenarios and may offer additional insights into this conundrum.

The epidemiologic study Block *et al.*[10] examined survival before the routine use of *sevelamer hydrochloride* and *paricalcitol*, that is, the two most commonly used medications for the management of osteodystrophy dialysis patients in the past few years.[11] Similarly, the default dialysate calcium concentration was 3.5 mEq/l in the past, but it has been reduced to 2.5 mEq/l in most MHD patients since early 2000s. A number of important questions about renal osteodystrophy have remained unanswered. It is not clear

whether a *fall* or *rise* in serum calcium or phosphorus over time has any association with subsequent survival independent of the baseline calcium or phosphorus values.

We studied a large and contemporary national cohort of MHD patients with repeated measures and with dialysate calcium concentration of 2.5 in over 80% of patients. We sought to examine whether the associations between surrogates of renal osteodystrophy and survival are similar in two different multivariate models: (1) time-dependent Cox models using time-varying repeated measures and (2) traditional Cox models with fixed covariates using only baseline values at the start of the cohort. We also examined the associations between changes in laboratory indices of renal osteodystrophy and death risk over time.

RESULTS

A total of 69 819 MHD patients were identified during the 2 years of observation. After deleting patients who did not remain beyond 3 months of MHD, that is, 5600 patients from the first seven quarters and 5870 patients from the last quarter, 58 349 MHD patients remained, of which 58 058 MHD patients had required data for the planned analyses. The latter group included 37 049 patients (64%) from the first calendar quarter data set (q1) and the rest from the subsequent quarters (q2–q8). Table 1 shows baseline demographic, clinical, and laboratory characteristics of the MHD patients during the first calendar quarter according to vitamin D analog administration status. Approximately 32% of the patients did not receive any vitamin D analog during the baseline quarter. A similar proportion was observed in subsequent calendar quarters (data not shown). Patients who did *not* receive a vitamin D analog were more likely to be Caucasians, to have slightly lower serum albumin and creatinine concentrations, and to receive lower doses of recombinant human erythropoietin (rHuEPO). The administered dialysate calcium concentration categories are not shown in Table 1 and included: 1.5 mEq/l or lower (0.7%), 2.0 mEq/l (3.5%), 2.5 mEq/l (84.2%), 3.0 mEq/l (7.8%), and 3.5% or higher (3.7%).

Table 2 shows correlation coefficients of serum calcium (not albumin adjusted) and phosphorus concentrations with relevant clinical and laboratory measures at the baseline of this cohort study. A higher serum albumin concentration was associated with a higher serum calcium level. Patients with higher serum phosphorus levels also had a higher serum creatinine and intact PTH but a lower serum bicarbonate concentration. However, most of these associations weakened substantially after multivariate adjustments.

Figures 1–4 show the association between the albumin-adjusted serum calcium, serum phosphorus, calcium–phosphorus product, intact PTH and alkaline phosphatase concentrations, and 2-year survival, respectively. Each figure consists of two panels that depict relative risks of all-cause mortality in *fixed* covariate (conventional) Cox models using only baseline data (upper panels) and time-dependent Cox models using time-varying repeated measure in up to eight calendar quarters (lower panel). The fixed-covariate models also include population frequency bar diagrams in the background.

Hypocalcemia was associated with an increased death risk in the unadjusted and case-mix adjusted models (reference calcium group: 9–9.5 mg/dl); however, controlling for MICS surrogates mitigated the association between low serum calcium and death substantially. In contrast, hypercalcemia

Table 1 | Baseline (first calendar quarter) data of 58 058 MHD patients (7/2001–6/2003), including 37 049 patients from the first quarter (q1) and 21 009 patients from subsequent quarters (q2–q8), divided into two groups based on the administration of vitamin D analog (paricalcitol or calcitriol) during the first calendar quarter

Variable	Received vitamin D at baseline[a] (*n*=39 305)	Did *NOT* receive vitamin D at baseline[a] (*n*=18 753)
Age (years)	60±15	61±16
Gender (% women)**	46	46
Diabetes mellitus (%)**	45	45
Race and ethnicity		
Caucasians (%)	32	48
Blacks (%)	38	20
Hispanics (%)*	18	17
Vintage (time on dialysis)		
3–6 months (%)	42	40
Primary insurance		
Medicare (%)**	60	60
Cohort time (days)	469±256	426±260
Body mass index (kg/m^2)**	26.4±6.3	25.9±5.6
Dialysate calcium (mEq/l)	2.55±0.28	2.58±0.28
K_t/V (single pool)**	1.53±0.31	1.54±0.32
nPCR or nPNA (g/kg/day)	1.0±0.2	1.0±0.2
Serum albumin (g/dl)	3.78±0.39	3.68±0.45
Creatinine (mg/dl)	9.3±3.2	8.5±3.3
TIBC (mg/dl)**	202±41	202±44
Ferritin (ng/ml)	610±493	602±530
Iron (ng/ml)**	61±26	60±27
Iron saturation ratio (%)	31±11	30±12
Bicarbonate (mEq/l)	21.7±2.7	22.0±2.9
Phosphorus (mg/dl)	5.8±1.4	5.5±1.7
Calcium (mg/dl)	9.3±0.7	9.2±0.8
Albumin adj. calcium (mg/dl)	9.3±0.7	9.2±0.8
Alkaline phosphatase (U/l)	115±86	112±94
Intact PTH (pg/ml)	374±362	228±319
Ca–Phos product (mg^2/dl^2)	53.8±13.6	50.5±15.9
Blood hemoglobin (g/dl)	12.0±1.3	11.9±1.3
WBC ($\times 10^3/\mu$l)	7.2±2.2	7.6±2.5
Lymphocyte (% of total WBC)	21±8	20±7
rHuEPO dose (U/week)	20 441±16 847	18 729±31 566

MHD, maintenance hemodialysis; nPCR, normalized protein catabolic rate; nPNA, normalized protein nitrogen appearance; PTH, parathyroid hormone; rHuEPO, recombinant human erythropoietin; TIBC, total iron-binding capacity; WBC, white blood cells.

[a]Baseline period of the cohort pertains to the first 3 months (first calendar quarter of the cohort)

**P*-value between 0.05 and 0.001, ** *P*-value > 0.05, all other *P*-values are < 0.001. All *P*-values for the difference between the two groups are < 0.001, unless specified.

Table 2 | Bivariate (unadjusted) and multivariate adjusted correlation coefficients between the quarterly averaged serum calcium and phosphorus concentrations and some relevant variables at baseline in 58 058 MHD patients

	Serum calcium		Serum phosphorus	
Variable	Pearson's correlation *r*	Multivariate adjusted correlation[a]	Pearson's correlation *r*	Multivariate adjusted correlation[a]
Age	−0.01**	+0.03	−0.32	+0.01**
K_t/V	+0.05	−0.01*	**−0.14**	−0.02
BMI	+0.02	−0.02	+0.09	−0.02
Serum albumin	**+0.31**	**+0.13**	**+0.17**	+0.05
nPCR	+0.01	+0.01**	**+0.23**	+0.02
TIBC	+0.11	+0.03	+0.08	+0.01
Ferritin	+0.05	+0.01	−0.05	−0.01**
Bicarbonate	+0.15	+0.06	**−0.35**	−0.02
Creatinine	+0.12	+0.04	**+0.42**	+0.05
Intact PTH	+0.01**	−0.06	**+0.33**	−0.02
Alkaline phosphatase	−0.07	+0.02	−0.01**	+0.01*
Blood hemoglobin	+0.11	+0.03	+0.03	+0.01*
WBC	−0.02	+0.04	+0.02	+0.01
Lymphocyte %	+0.09	+0.02	+0.02	+0.01**
Administered rHuEPO dose	−0.07	+0.01**	+0.04	+0.01**
Paricalcitol dose	+0.15	+0.01**	**+0.17**	−0.03
Calcitriol dose	−0.01**	−0.01**	−0.01	−0.01**

BMI, body mass index; MHD, maintenance hemodialysis; nPCR, normalized protein catabolic rate; PTH, parathyroid hormone; rHuEPO, recombinant human erythropoietin; TIBC, total iron-binding capacity; WBC, white blood cells.
[a]Multivariate models include all case-mix and MICS covariates.
**P*-value >0.05; **P*-value between 0.01 and 0.05; all other *P*-values are <0.01.
All *P*-values are <0.01 unless specified. Correlation coefficients >0.10 are in bold.

(>8.5 mg/dl in the fixed-covariate model and >10.5 mg/dl in the time-dependent model) continued to remain a strong predictor of incrementally higher death risk (see Figure 1). Similarly, hypophosphatemia was a strong correlate of death high risk in both unadjusted and case-mix adjusted models (reference phosphorus group: 5–6 mg/dl), but a large proportion of this association was mitigated after controlling for MICS. Hyperphosphatemia (>6 mg/dl) was a strong correlate of higher death risk in both models even after case-mix and MICS adjustment (Figure 2). The calcium–phosphorus product showed a similar trend with incrementally higher death risks in multivariate adjusted models (reference group: 45–50 mg^2/dl^2, see Figure 3).

In unadjusted models, only lower values of serum intact PTH (<200 pg/ml) were associated with mortality, whereas mortality risk of higher values (>300 mg/dl) did not differ from the reference group of 200–300 pg/ml (see Figure 4). However, multivariate adjustments disclosed a strong association between incrementally higher serum PTH values and increased death risk. The association between serum alkaline phosphatase (usual normal range: 25–150 u/l) and death risk was more monotonic. Compared to the selected reference group of 70–80 U/l, incrementally higher alkaline phosphatase values exhibited a relatively linear association with higher death risks (Figure 5).

In order to investigate whether changes over time in serum calcium, phosphorus and the calcium–phosphorus product beyond Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines recommended ranges are associated with changes in death risk independent of their baseline values or other clinical or laboratory measures, Cox regression modeling for these changes was examined in those patients whose baseline values were within the K/DOQI recommended ranges,[12] that is, baseline albumin-adjusted serum calcium between 8.4 and 9.5 mg/dl ($n = 17\,113$), baseline serum phosphorus between 3.5 and 5.5 mg/dl ($n = 13\,184$) and baseline calcium–phosphorus product <55 mg^2/dl^2 ($n = 18\,423$) (Figure 6). An excessive fall (or rise) in serum calcium greater than 0.6 mg/dl in 6 months was associated with higher death risk in patients whose baseline value was within the K/DOQI recommended calcium range (Figure 6, upper panel). A similar U-curve association with mortality was also observed for excessive changes in serum phosphorus (Figure 6, middle panel). An incremental rise in serum calcium–phosphorus product beyond 10 mg^2/dl^2 per 6 months was also associated with progressively increasing death risks in MHD patients whose baseline serum calcium–phosphorus product was within the K/DOQI recommended range, that is, <55 mg^2/dl^2 (Figure 6, lower panel).

In order to examine the association between the administration of paricalcitol and survival, we *a priori* classified MHD patients into five groups according to the average dose of paricalcitol administered within each calendar quarter of the cohort after excluding those who received calcitriol (Table 3). Patients who did not receive paricalcitol or received

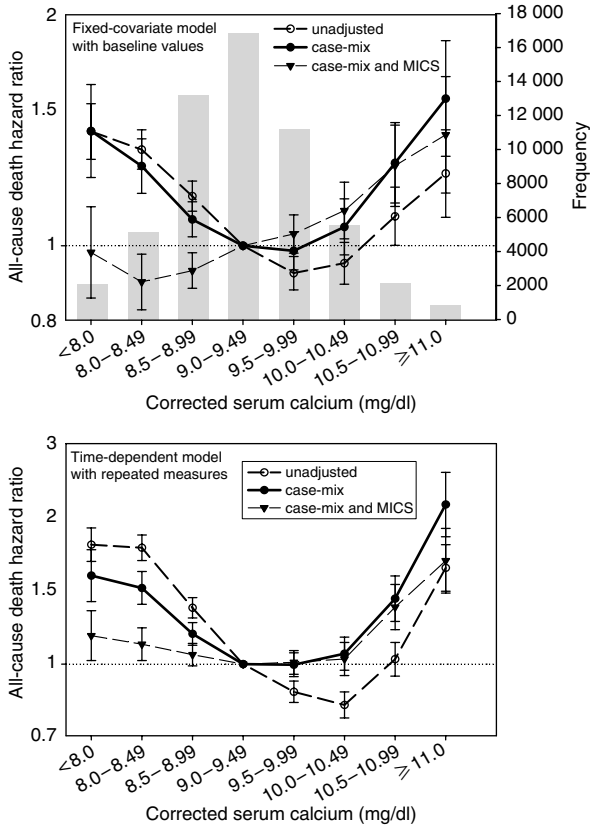

Figure 1 | Association between albumin-adjusted serum *calcium* values and the relative risk of death in 58 058 MHD patients over a 2-year interval (July 2001–June 2003) using fixed-covariate Cox modeling with only baseline values (upper panel) and time-dependent Cox models with time-varying repeated measures (lower panel). The fixed-covariate model also includes a background bar diagram to reflect patient population frequency in each group.

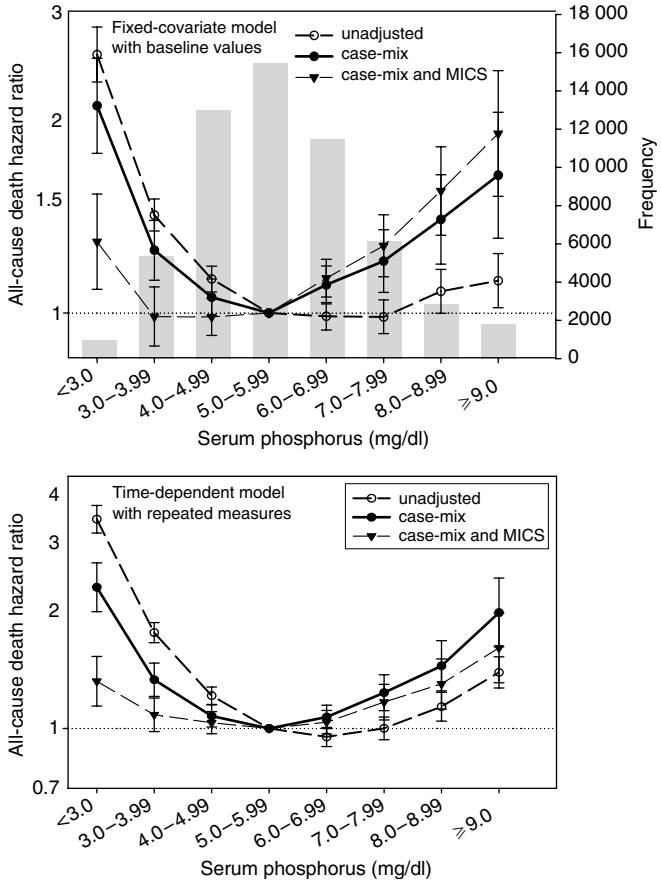

Figure 2 | Association between the time-varying serum *phosphorus* values and the relative risk of death in 58 058 MHD patients over a 2-year interval (July 2001–June 2003) using fixed-covariate Cox modeling with only baseline values (upper panel) and time-dependent Cox models with time-varying repeated measures (lower panel). The fixed-covariate model also includes a background bar diagram to reflect patient population frequency in each group.

low doses (between 1 and 5 μg/week) had the lowest baseline serum PTH, whereas those who received incrementally higher paricalcitol doses had higher baseline serum PTH concentrations (Table 3). Figure 7 shows the association between paricalcitol dose and mortality risk in 55 716 MHD patients, that is, after excluding 2342 patients who received calcitriol at least once during any given quarter. Receiving any dose of paricalcitol was associated with greater survival as compared to those who did not receive paricalcitol. However, among those who received paricalcitol, those who required incrementally higher doses showed a trend toward increased death risk (Figure 7).

DISCUSSION

In a 2-year cohort of 58 058 MHD patients from one large dialysis organization across the USA at the dawn of the twenty-first century, we examined both fixed baseline and time-varying associations between several factors related to renal osteodystrophy and survival. Our findings include associations that are both confirmatory of findings by other investigators[6–11,13] and novel associations that have not been reported previously. These findings may further support K/DOQI recommendations for the management of renal osteodystrophy.[12]

Serum minerals and mortality

Higher serum calcium and phosphorus levels were consistently associated with increased death risks, which is consistent with recent findings by Block *et al.*[7,10] and other investigators who used non-time-dependent models.[14,15] In time-dependent models, higher a serum calcium threshold (>10.5 mg/dl) was associated with increased death risk, whereas in non-time-dependent models, the mortality predictability of hypercalcemia starts at lower calcium level (>8.5 mg/dl). Additional studies are needed to ascertain the clinical relevance of such differences.

We also found that some of the naive associations traditionally observed between lower serum calcium and phosphorus levels and higher mortality risks[16,17] may be owing to the confounding effect of MICS and its association with outcome (Figure 1). The pronounced association between hypophosphatemia and death (Figure 2) can be

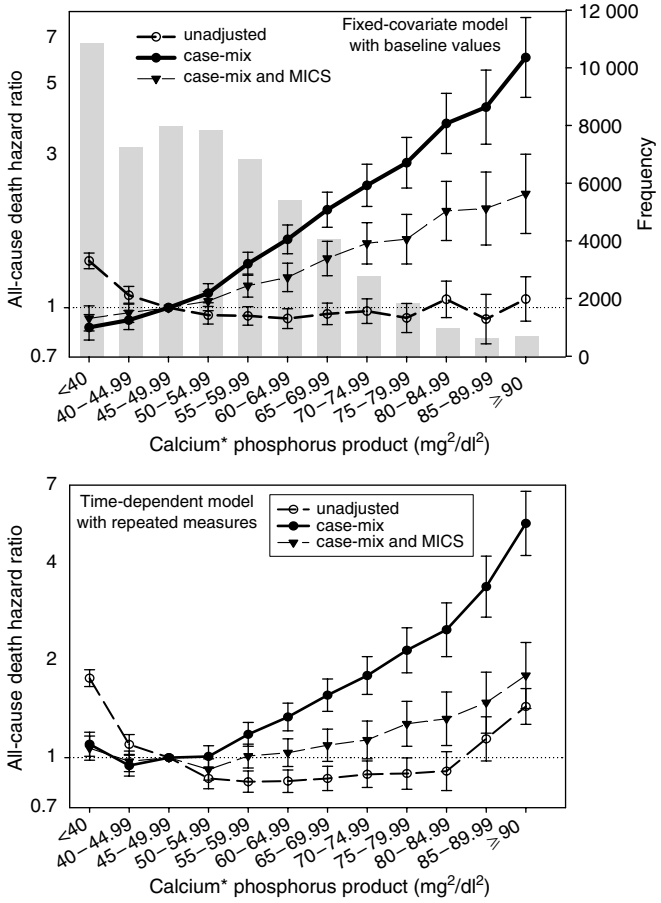

Figure 3 | Association between the time-varying *product of serum calcium and phosphorus* values and the relative risk of death in 58 058 MHD patients over a 2-year interval (July 2001–June 2003) using fixed-covariate Cox modeling with only baseline values (upper panel) and time-dependent Cox models with time-varying repeated measures (lower panel). The fixed-covariate model also includes a background bar diagram to reflect patient population frequency in each group.

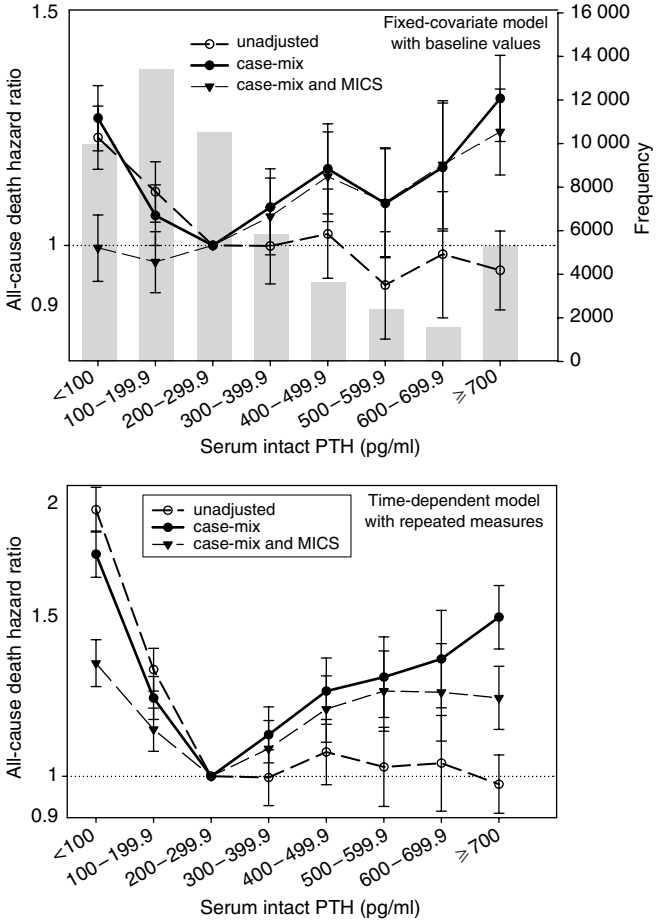

Figure 4 | Association between the time-varying serum *intact PTH* values and the relative risk of death in 58 058 MHD patients over a 2-year interval (July 2001–June 2003) using fixed-covariate Cox modeling with only baseline values (upper panel) and time-dependent Cox models with time-varying repeated measures (lower panel). The fixed-covariate model also includes a background bar diagram to reflect patient population frequency in each group.

an indicator of poor nutritional intake.[17,18] In contrast, hyperphosphatemia remained a strong predictor of poor survival even after MICS adjustment, consistent with previous findings.[10,15] The underlying association between high calcium–phosphorus products, which was almost entirely masked by case-mix characteristics of the patients (Figure 3), was found to be monotonic and strictly up-going after multivariate adjustments, similar to what that has been reported previously using non-time-dependent models.[10,15] In addition to examining the time-dependent associations between the measured serum concentrations of the minerals and mortality, we also found that *changes* over time in these measures may have a bearing on survival (Figure 6). These findings may verify the appropriateness of the K/DOQI recommended ranges.

Serum PTH and survival

The lack of any association between high ranges of serum PTH and mortality in MHD patients was masked almost entirely by the case-mix characteristics of the patients (Figure 4); this is consistent with a recent database analysis by Block *et al.*[10] If this association is causal, it may explain why vitamin D analogs that lower the PTH level are associated with better survival as reported by Teng *et al.*[13] and also observed by ourselves in the current study. We also found that lower levels of serum PTH, especially below the K/DOQI recommended lower threshold (<150 pg/ml), are associated with increased death risk (see Figure 4) as indicated by others.[19,20]

Serum alkaline phosphatase and mortality

In this study, the association between serum alkaline phosphatase and survival was also examined. This monotonic and almost strictly up-going association independent of the level of multivariate adjustment (Figure 5) is in sharp contrast to the associations between minerals or PTH and survival, which are U or J shaped (Figures 1–4). The K/DOQI guidelines states that the deleterious effects of high serum

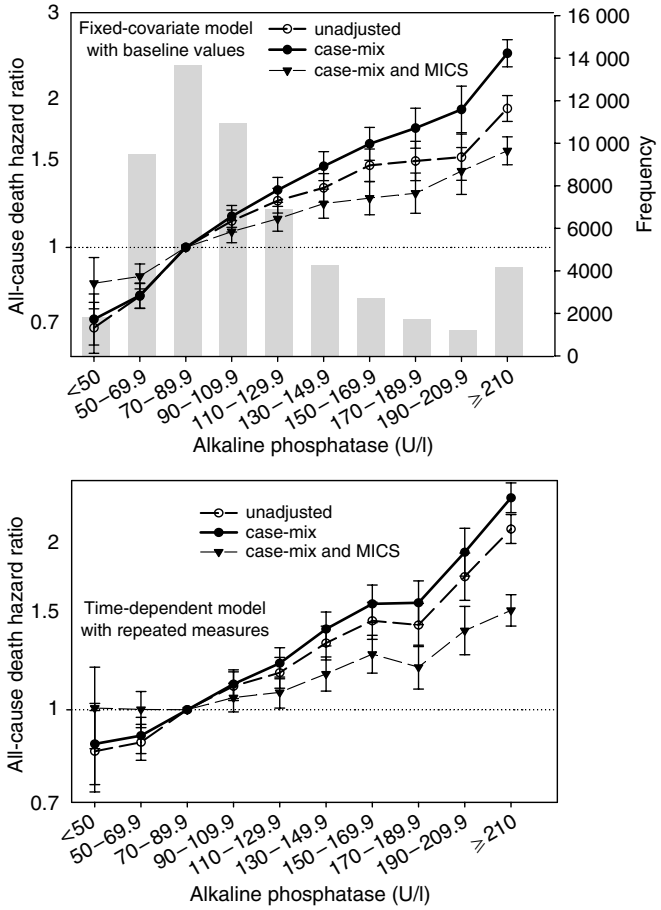

Figure 5 | Association between the time-varying serum *alkaline phosphatase* values and the relative risk of death in 58 058 MHD patients over a 2-year interval (July 2001–June 2003) using fixed-covariate Cox modeling with only baseline values (upper panel) and time-dependent Cox models with time-varying repeated measures (lower panel). The fixed-covariate model also includes a background bar diagram to reflect patient population frequency in each group.

PTH levels may be manifested by elevated bone alkaline phosphatase activity owing to associated bone resorption.[12] To that end, the correlation between alkaline phosphatase and PTH was 0.27 in our study but much lower with serum calcium ($r = 0.09$) and phosphorus concentration ($r = 0.06$). However, the inclusion of PTH in the multivariate model did not mitigate the monotonic association with death (data not shown).

It is important to note that the alkaline phophatase measured routinely in dialysis patients is not bone-specific. Liver disease may be associated with increased serum alkaline phosphatase level. Indeed, we found a moderate association between alkaline phosphatase and the liver enzyme serum glutamic oxaloacetic transaminase (aspartate aminotransferase) in our study ($r = +0.29$). However, even though liver diseases such as hepatitis C are associated with increased liver enzyme and with increased death risk in MHD patients,[21] a large proportion of the mortality predictability of alkaline phosphatase is likely owing to renal osteodystrophy.

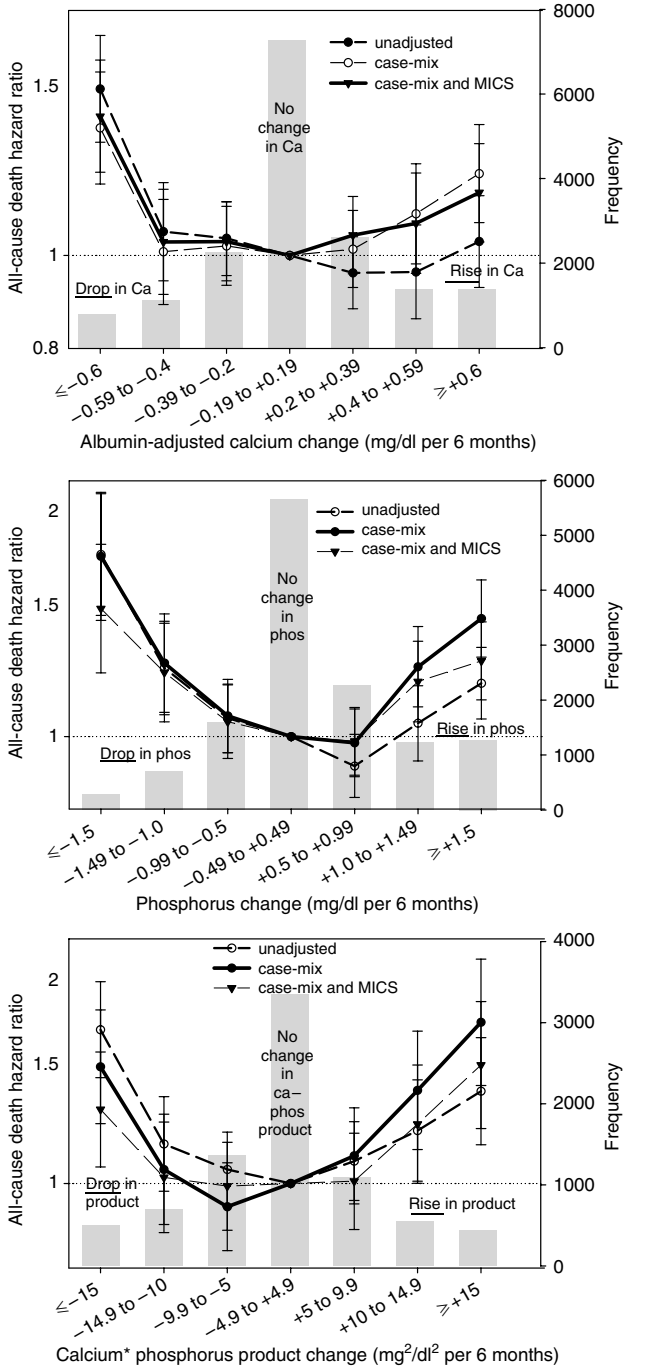

Figure 6 | Association between the *changes* in albumin-adjusted serum calcium (upper panel), serum phosphorus (middle panel), and the product of serum calcium and phosphorus (lower panel) during the first 6 months of the cohort (July 2001–12/2001) and the subsequent 18-month (1/2002–June 2003) risk of all-cause death in MHD patients. Note that the selected patients in each panel are those with a baseline value within the K/DOQI recommended range,[12] that is, the baseline serum calcium value of patients in the upper panels in between 8.4 and 9.5 mg/dl ($n = 17\,113$), the baseline serum phosphorus value of patients in the middle panel is between 3.5 and 5.5 mg/dl ($n = 13\,184$), and the baseline serum Ca–Phos product of patients in the lower panel is < 55 mg^2/dl^2 ($n = 18\,423$). Each panel also includes a background bar diagram to reflect patient population frequency in each group.

Table 3 | Selected categories of administered paricalcitol and 2-year mortality census [rate] among 58 058 MHD patients

Paricalcitol (μg/week)	Group size (%)	All-cause death [%]	Cardio-vascular death [%]	Baseline calcium (mg/dl)	Baseline phosphorus (mg/dl)	Baseline PTH (pg/ml)	Baseline albumin (g/dl)
None given	20 663 (36)	5459 [26]	2196 [11]	9.2 (0.7)	5.5 (1.7)	238 (323)	3.69 (0.44)
1–4.9	5288 (9)	1326 [25]	558 [11]	9.2 (0.6)	5.4 (1.5)	229 (222)	3.73 (0.41)
5–9.9	11 965 (21)	2960 [25]	1346 [12]	9.2 (0.7)	5.6 (1.5)	277 (231)	3.76 (0.39)
10–14.9	8326 (14)	1998 [24]	878 [11]	9.3 (0.7)	5.9 (1.4)	358 (296)	3.77 (0.39)
⩾15.0	11 816 (20)	2786 [24]	1265 [11]	9.5 (0.8)	6.2 (1.4)	555 (476)	3.81 (0.37)

MHD, maintenance hemodialysis; PTH, parathyroid hormone.
Continuous values are in form of (mean ± s.d.).

Paricalcitol and survival

This study also confirms the recently reported association between the administration of any dose of paricalcitol and greater survival in MHD patients (Figure 7).[13,22] In some models and across some but not all paricalcitol dose groups, requiring higher doses was associated with a trend toward higher death risk (Figure 7). This inconsistent trend might be owing to a higher baseline serum PTH level among those who were administered higher paricalcitol doses.[23,24] This association may be somewhat analogous to what has been described for rHuEPO in MHD patients, in whom requiring higher doses of rHuEPO are associated with higher death risk,[25,26] possibly owing to the association of rHuEPO resistance with inflammation.[27]

Strength and limitation of the study

Our study should be qualified because it is observational, rather than interventional, and because a mixed incident/prevalent MHD population was examined. Moreover, the impact of therapy with sevelamer on renal osteodystrophy cannot be examined by these analyses.[28] Nevertheless, as essentially all MHD patients of the DaVita dialysis facilities were included in our analyses, the likelihood of selection bias is minimal. Moreover, all dialysis facilities were under uniform administrative care, and all laboratory tests were performed in one single laboratory with optimal quality assurance monitoring. Furthermore, we used 3-month averaged measures rather than one single measure at baseline, and we adjusted for dialysis vintage in all multivariate models.

Similar to the study by Teng *et al.*[13] as well as our previous studies,[29–32] we did not include history of cardiovascular or other comorbidities as covariates. However, diabetes data were available and adjusted for in all multivariate models. Moreover, many case-mix and MICS covariates that were included in the models are known to have strong associations with comorbid conditions. The limited comorbidity data used in some previous studies usually originated from the dialysis initiation form (Form 2728), in which comorbid conditions are significantly underreported[33] and which is outdated for prevalent patients with higher vintage periods. Another limitation of our study is lack of explicit laboratory markers of inflammation such as C reactive protein. However, we did use data on serum albumin, ferritin, and total iron binding capacity and blood WBC and rHuEPO dose, all of which have known associations with inflammation. Finally, the studied cohort was before the calcimimetic agent *cinacalcet* was approved by the Food and Drug Administration.[34] Hence, the relationship between osteodystrophy and survival may be altered in patients receiving *cinacalcet* starting 2004.

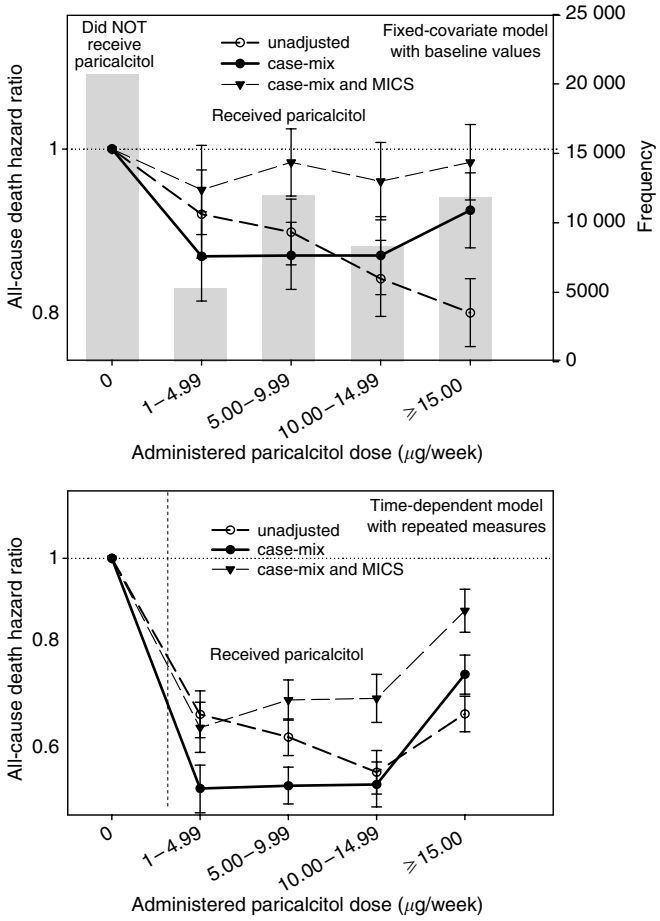

Figure 7 | Association between the time-varying administered dose of paricalcitol and the relative risk of death in 58 058 MHD patients over a 2-year interval (July 2001–June 2003) using fixed-covariate Cox modeling with only baseline values (upper panel) and time-dependent Cox models with time-varying repeated measures (lower panel). The fixed-covariate model also includes a background bar diagram to reflect patient population frequency in each group.

Conclusions and clinical implications

Whereas both time-dependent and fixed-covariate Cox models resulted in similar associations between osteodystrophy indicators and survival in many instances, subtle but potentially clinically relevant differences between the two models existed, probably because fixed models do not account for variations of osteodystrophy indices and changes in medication dose over time. Hence, time-dependent models may be clinically more relevant, as they simulate the real clinical scenarios, in which physicians evaluate and manage the MHD patients.

Adjustment for MICS resulted in changes in the magnitude and direction of some but not all associations. Although such extensive multivariate adjustments may be indicated, they may also introduce overadjustment bias,[35,36] because there is a strong collinearity between MICS markers and osteodystrophy surrogates (see Table 2) and because MICS markers may be in the causal pathways that link osteodystrophy to outcomes.[37,38] Hence, whereas case-mix adjustments appear appropriate, we caution against interpretation of the associations after additional MICS adjustment. Moreover, bias by indication may also have led to patients with different death risk profiles being treated differently, for example, patients with higher death risk may not have received vitamin D analogs, leading to an apparent association between vitamin D administration and better survival. More studies including randomized trials are needed to verify these associations.

MATERIALS AND METHODS

Patients

We examined prospectively collected data of a 2-year (1 July 2001–30 June 2003) historical cohort of all MHD patients from virtually all DaVita Inc., dialysis facilities in the USA, the then second largest dialysis care provider in the nation. The database creation has been described elsewhere.[29,30,32,39] This database included information on approximately 40 000 maintenance dialysis patients at any given time. All repeated measures for each patient within a given calendar quarter (13-week interval) were averaged to obtain one *quarterly* mean value and to mitigate the effect of short-term variations. Hence, up to eight repeated and quarterly varying values were available for each measure in each MHD patient over a 2-year observation period. The study was approved by the Institutional Review Committees of Harbor-UCLA and DaVita Inc.

Dialysis vintage was defined as the duration of time between the first day of maintenance dialysis treatment anywhere and the first day that the patient entered the cohort under the study. The entry quarter was defined as the first calendar quarter in which patient's dialysis vintage was greater than 3 months for at least half of the duration of the quarter. By implementing this criterion, any patient who did not remain in the cohort beyond the first 3 months of MHD was excluded.

Laboratory methods

All laboratory measurements were performed by DaVita Laboratories in Deland, FL, USA using standardized and automated methods. For each laboratory measure, the mathematical average of all available values within any given calendar quarter (13-week intervals) was calculated and used in all analyses. Most blood samples were collected pre-dialysis with the exception of the post-dialysis serum urea nitrogen, which was obtained in order to calculate urea kinetics. The normalized protein nitrogen appearance, also known as protein catabolic rate, and K_t/V were calculated using urea kinetic modeling formulas.[40,41] Blood samples were drawn using uniform techniques in all DaVita dialysis clinics across the nation and were transported to the DaVita Laboratory in Deland, Florida, USA, usually within 24 h of collection. Most laboratory values, including complete blood cell counts and serum levels of urea nitrogen, albumin, creatinine, calcium, phosphorus, bicarbonate, iron, and total iron-binding capacity, were measured monthly. Serum intact PTH (first-generation immunoradiometric PTH assay, Nichols, San Juan Capistrano, CA, USA; Nussbaum *et al.*[42]) and ferritin was usually measured at least once during each calendar quarter. Both normalized protein nitrogen appearance and K_t/V were calculated monthly. Hemoglobin was measured weekly to bi-weekly in most patients. Serum calcium was adjusted for serum albumin according to the following equation: adjusted calcium = measured calcium + ((4.0 − serum albumin in g/dl) × 0.8).

Laboratory measures of osteodystrophy were stratified *a priori* similar to what was previously carried out by Block *et al.*[10]: (1) albumin-adjusted serum calcium was divided into eight categories of <8, $\geqslant 11$ mg/dl, and six 0.5 g/dl groups in-between; (2) serum phosphorus into eight categories of <3, $\geqslant 9$ mg/dl, and six 0.5 g/dl in-between; (3) the product of serum phosphorus and calcium into 12 categories of <40, $\geqslant 90$, and ten 10 mg^2/dl^2 integers in-between; (4) serum intact PTH into eight categories of <100, $\geqslant 700$ pg/ml and six 100 pg/ml groups in-between; and (5) serum alkaline phosphatase into 10 categories of <50, $\geqslant 210$ U/l, and eight 20 U/l in-between.

The following nine time-varying (quarterly changing) laboratory variables with up to eight repeated measures per patient over the 2-year cohort time and with known associations with survival in MHD patients were also included in the time-dependent Cox models as time-varying surrogates of MICS: (1) serum albumin, (2) normalized protein nitrogen appearance or normalized protein catabolic rate, (3) serum total iron-binding capacity, (4) serum creatinine, (5) serum bicarbonate, (6) serum ferritin, (7) blood hemoglobin, (8) peripheral white blood cell count (WBC), and (9) lymphocyte percentage.

Administered in-center medications

The averaged doses of the in-center (in dialysis facility) administered vitamin D analogs *paricalcitol* (Zemplar™) and *calcitriol* (Calcijex, both from Abbott Laboratories, Abbott Park, IL, USA), which are related to the management of renal osteodystrophy, were also calculated in μg/week for each calendar quarter and included in all case-mix adjusted models as additional time-varying covariates. Paricalcitol *per se* was also examined as an independent predictor of outcome in separate models (see below). The dose of administered rHuEPO (EPOGEN, Amgen Inc., Thousand Oaks, CA, USA) was also calculated for each calendar quarter in units/week and included in the MICS-adjusted models as an indicator of inflammation.[27,43,44] To examine the association between the administered paricalcitol and survival in MHD patients, five groups of paricalcitol dose/status were created *a priori* after excluding MHD patients who received calcitriol or other vitamin D analogs ($<4\%$ together): (1) those who did not receive any paricalcitol during the entire 13 weeks of a given quarter; (2) those who received paricalcitol between 1 and 4.9 μg/week; (3) paricalcitol between 5 and 9.9 μg/week; (4) paricalcitol

between 10 and 14.9 μg/week; and (5) paricalcitol of 15 μg/week or greater.

Statistical and epidemiologic methods

For every time-varying measure, up to eight independent quarterly values were obtained in each patient. Both fixed-covariate and time-dependent Cox proportional hazard regression analyses (PROC PHREG)[45] were conducted to examine whether the 2-year survival was associated with surrogates of bone disease. A hazard ratio greater than 1.00 indicates increased death risk and below 1.00 implies improved survival chance. A 95% confidence interval surpassing 1 is considered insignificant.

For each analysis, three types of models were examined based on the level of multivariate adjustment: (1) *unadjusted* models included indicators of osteodystrophy (calcium, phosphorus, calcium–phosphorus product, intact PTH, or alkaline phosphatase) as the predicting variable, entry quarter as the covariate, and mortality as the outcome variable; (2) *case-mix adjusted* models included additional covariates: age, gender, race and ethnicity, diabetes mellitus, vintage, primary insurance, marriage status, and standardized mortality ratio of the dialysis clinic during entry quarter, continuous values of K_t/V, dialysate calcium concentration, and administered doses of each of vitamin D analogs within each calendar quarter; and (3) *case-mix and MICS adjusted* models included all of the above-mentioned covariates plus 11 indicators of nutritional status and inflammation including the time-varying body mass index, averaged dose of rHuEPO in each calendar quarter, and the nine above-mentioned time-varying laboratory values (see above under Laboratory methods). All laboratory markers, vitamin D analogs and rHuEPO dose, K_t/V, and body mass index were included as *time-varying* covariates with up to eight independent quarterly values per variable per each patient. When paricalcitol dose was modeled as the predicting variable, time-varying serum calcium, phosphorus, and intact PTH concentrations were also included as additional case-mix covariates. Missing covariate data (0 to $<2\%$ for all variables, except for serum ferritin, intact PTH, and body mass index) were imputed by the mean or median of the existing values, whichever was most appropriate. For the three variables with $>2\%$ missing values, a dummy variables was created to indicate the missing status. All descriptive and multivariate statistics were carried out with the SAS, version 8.02, SAS Institute Inc., Cary, NC, USA. Owing to the large sample size, most *P*-values tend to be small.

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