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Serum Alkaline Phosphatase Predicts Mortality among Maintenance Hemodialysis Patients

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ABSTRACT

Several observational studies have demonstrated that serum levels of minerals and parathyroid hormone (PTH) have U- or J-shaped associations with mortality in maintenance hemodialysis patients, but the relationship between serum alkaline phosphatase (AlkPhos) and risk for all-cause or cardiovascular death is unknown. In this study, a 3-yr cohort of 73,960 hemodialysis patients in DaVita outpatient dialysis were studied, and the hazard ratios for all-cause and cardiovascular death were higher across 20-U/L increments of AlkPhos, including within the various strata of intact PTH and serum aspartate aminotransferase. In the fully adjusted model, which accounted for demographics, comorbidity, surrogates of malnutrition and inflammation, minerals, PTH, and aspartate aminotransferase, AlkPhos ≥120 U/L was associated with a hazard ratio for death of 1.25 (95% confidence interval 1.21 to 1.29; $P < 0.001$). This association remained among diverse subgroups of hemodialysis patients, including those positive for hepatitis C antibody. A rise in AlkPhos by 10 U/L during the first 6 mo was incrementally associated with increased risk for death during the subsequent 2.5 yr. In summary, high levels of serum AlkPhos, especially >120 U/L, are associated with mortality among hemodialysis patients. Prospective controlled trials will be necessary to test whether serum AlkPhos measurements could be used to improve the management of renal osteodystrophy.

In advanced chronic kidney disease (CKD; stages 3 through 5), secondary hyperparathyroidism (SHPT), along with renal osteodystrophy, is common and may be associated with abnormal mineral metabolism and/or abnormal serum or tissue mineral levels, vascular calcifications, and poor survival, espe-

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cially among those who undergo maintenance dialysis treatment.[1–4] Two recent epidemiologic studies using national databases of the two large dialysis organizations in the United States showed associations between measures of renal osteodystrophy and mortality in maintenance hemodialysis (MHD) patients.[1,2] The National Kidney Foundation Kidney Disease Outcome Quality Initiative (KDOQI) Guidelines on CKD Mineral and Bone Disorders (MBD) includes a comprehensive review of the literature along with clearly recommended target ranges for serum calcium, phosphorus, calcium-phosphorus product, and parathyroid hormone (PTH) concentrations in patients with CKD[5]; however, these KDOQI guidelines provide rather limited discussion with no target range for the desired serum alkaline phosphatase (AlkPhos) level, which is measured routinely (usually at least once monthly) in virtually all dialysis patients in the United States.

Serum AlkPhos is a biochemical marker of bone turnover and is used to monitor the metabolic bone disease associated with renal insufficiency.[6] Unlike serum PTH that originates from the parathyroid glands and affects bone metabolism,[3,4,7] serum AlkPhos stems from the bone itself and reflects internal bone activities.[6] Incrementally higher levels of serum AlkPhos can be seen with worsening magnitude of bone turnover.[8,9] We recently showed that surrogates of CKD-MBD, including higher serum levels of minerals and higher AlkPhos levels, were associated with increased all-cause death risk in a 2-yr cohort of 58,000 MHD patients.[2] A recent meta-analysis showed that a decline in serum AlkPhos, not PTH, was the most consistent result of the active vitamin D administration in patients with CKD.[10] In this study, however, adjustment for comorbid states or liver function tests was not implemented. Serum AlkPhos may also increase as a result of liver disorders[2]; therefore, it is unclear whether the foregoing association between AlkPhos and survival is related to liver disorders. Moreover, it is not known whether the association between serum AlkPhos and mortality holds at different strata of PTH levels.

In this study, examining a 3-yr cohort of approximately 74,000 MHD patients, we hypothesized that serum AlkPhos is independently associated with both all-cause and cardiovascular mortality after adjustment for potential confounders, including comorbid states, markers of liver function, and serum PTH. We also hypothesized that this association persists in diverse subgroups of MHD patients. In particular, we hypothesized that the mortality predictability of serum AlkPhos is independent of the various ranges of serum PTH or serum liver enzyme measurements. We also examined the effect of changes in serum AlkPhos during the first 6 mo of the cohort on subsequent patient survival.

RESULTS

The original 3-yr (July 2001 through June 2004) national database of all DaVita MHD patients included 102,255 cumulative patients. After deleting patients who did not maintain be-

yond 45 d of HD treatment (13,657 patients from the first 11 calendar quarters and 5348 patients from the last quarter), 83,250 MHD patients remained for analysis. The latter included 37,386 (45%) patients from the first calendar quarter data set (Q1) and the rest from the subsequent calendar quarters (Q2 through Q12). A total of 73,960 MHD patients had documented serum AlkPhos values.

Table 1 compares 51,367 with a baseline (3-mo averaged) serum AlkPhos value <120 U/L *versus* 22,593 patients with values ≥120 U/L, which is the upper limit of normal range. Patients with higher serum AlkPhos concentrations were 3 yr younger, included more women and patients with diabetes, and had higher serum AST and intact PTH (iPTH) values. As shown in Table 2, serum AlkPhos had the strongest correlations with AST and iPTH. Figure 1 shows averaged serum AlkPhos concentrations in selected strata of serum iPTH and AST. Patients with iPTH >600 pg/ml or AST >30 U/L were more likely to have abnormally higher AlkPhos >120 U/L.

To examine further the association between increments of serum AlkPhos and survival, we divided AlkPhos values into eight *a priori* selected categories (<60 to ≥180 U/L with 20-U/L increments in between). As shown in Table 3, almost 70% of the patients had a baseline AlkPhos <120 U/L. Using the AlkPhos range of 80 to 100 U/L as the reference group (because of its largest sample size and its high event rates), the unadjusted and multivariate adjusted all-cause and cardiovascular death rates showed a strictly upward trend with increasing AlkPhos levels (Figure 2A). A similar association was also noticed with cardiovascular mortality (Figure 2B). Additional sensitivity analyses using time-dependent models that account for quarterly changing serum AlkPhos and other laboratory values based on one calendar quarter lag (Figure 2C, Table 4) or two calendar quarter lag (Figure 2D) showed similar associations for high AlkPhos levels, whereas low levels were not significantly associated with greater survival.

For examination of whether a fall or a rise in AlkPhos over time has any bearing on subsequent mortality, MHD patients who had measured AlkPhos over the first two consecutive calendar quarters (Q1 to Q2) were divided into three categories of unchanged (if the magnitude of AlkPhos change from Q1 to Q2 remained between −10 and 10 U/L), increased, and decreased AlkPhos during 6 mo. The last two categories were each further subdivided into three subgroups of 10-U/L change in AlkPhos during 6 mo (Table 5). For mitigation of the likelihood of the regression to the mean, especially for the outliers, only patients whose baseline serum AlkPhos was between 50 and 200 U/L were included in this analysis. Furthermore, multivariate survival models were adjusted for baseline AlkPhos. As shown in Figure 3, a rise in AlkPhos during the first 6 mo was incrementally associated with increasing death risk in the subsequent 2.5 yr, but an expected reciprocal association between a drop in AlkPhos and greater survival was not clearly evident.

We found that in the fully adjusted model, an abnormally high AlkPhos ≥120 U/L was associated with a death hazard ratio of 1.25 (95% confidence interval [CI] 1.21 to 1.29; *P* <

Table 1. Baseline calendar quarter data of 73,960 MHD patients according to the threshold level of serum AlkPhos of 120 U/L[a]

Variable	AlkPhos <120 U/L (n = 51,367)	AlkPhos ≥120 U/L (n = 22,593)
Age (yr; mean ± SD)	62 ± 16	59 ± 15
Gender (% women)	44	52
Diabetes (%)	45	51
Race/ethnicity (%)		
white	40	35
black[b]	32	33
Hispanic	14	18
Vintage (time on dialysis; %)		
<6 mo	32	29
6 to 24 mo	18	14
2 to 5 yr[c]	21	22
≥5 yr	28	36
Primary insurance		
Medicare (%)[c]	65	65
Known causes of death		
cardiovascular (% of all-cause)	52	49
infectious (% of all-cause)	12	14
Hepatitis C antibody positivity (%)[d]	10	15
BMI (kg/m²; mean ± SD)	26.6 ± 6.2	25.8 ± 6.2
Kt/V (single pool; mean ± SD)[c]	1.5 ± 0.3	1.5 ± 0.3
Protein intake (nPCR or nPNA; g/kg per d; mean ± SD)[c]	1.0 ± 0.3	1.0 ± 0.3
Serum AST (U/L; mean ± SD)	17 ± 9	23 ± 20
albumin (g/dl; mean ± SD)	3.78 ± 0.40	3.61 ± 0.49
creatinine (mg/dl; mean ± SD)	8.9 ± 3.4	8.2 ± 3.3
ferritin (ng/ml; mean ± SD)	442 ± 284	501 ± 311
TIBC (mg/dl; mean ± SD)	205 ± 43	199 ± 47
bicarbonate (mg/dl; mean ± SD)	21.9 ± 2.9	21.7 ± 3.1
phosphorus (mg/dl; mean ± SD)	5.7 ± 1.5	5.6 ± 1.6
calcium (mg/dl; mean ± SD)	9.3 ± 0.7	9.1 ± 0.8
iPTH (pg/ml; mean ± SD)	209 ± 115	273 ± 199
Blood hemoglobin (g/dl; mean ± SD)	12.1 ± 1.3	11.9 ± 1.4
WBC (×10³/μl; mean ± SD)	7.3 ± 2.3	7.5 ± 2.7
lymphocyte (% of total WBC; mean ± SD)	21 ± 8	20 ± 8

[a]BMI, body mass index; nPCR, normalized protein catabolic rate; nPNA, normalized protein equivalent of total nitrogen appearance; WBC, white blood cell count. All P values are <0.001 for the difference between the two groups, unless otherwise specified.
[b]$P \geq 0.001$ and ≤ 0.05.
[c]$P \geq 0.05$.
[d]Hepatitis C antibody data were available only for 18% of the cohort (n = 13,664).

0.001). For examination of whether this association is consistently observed across different groups of MHD patients, the mortality predictability of AlkPhos ≥120 U/L was studied within diverse patient subgroups as shown in Figure 4. The death risk of high AlkPhos was found to be increased across all of these groups, including in both hepatitis C virus (HCV) antibody–positive and –negative patients, in 13,664 MHD patients whose HCV status was tested.[11] Additional analyses were performed for the three strata of serum iPTH, consistent with the KDOQI recommended target zone (<150 between 150 and 300, and ≥300 pg/ml; Figure 5), as well as two selected strata of AST (<30 and ≥30 U/L; Figure 6). As shown in Figures 5 and 6, the associations between increasing AlkPhos and 3-yr death risk remained consistent across the foregoing PTH and AST strata. Additional sensitivity analyses in the HCV antibody

strata showed similar results (data not shown). Inclusion of interaction terms, including PTH × AlkPhos, had no meaningful impact of found associations (data not shown). For achievement of a more commensurate analysis, mortality predictability of serum AlkPhos and iPTH was compared across their deciles in two separate fully adjusted, non–time-dependent models (Figure 7). The AlkPhos–mortality association seemed somewhat linear, whereas the PTH–mortality association was U-shaped.

DISCUSSION

We found that in 73,960 MHD patients from a large dialysis organization in the 21st century, higher AlkPhos values were

Table 2. Bivariate (unadjusted) and partial (adjusted) correlation coefficients between serum AlkPhos and selected baseline variables in 73,960 MHD patients[a]

Variable	Bivariate Correlation	Adjusted Correlation
Age	−0.09	−0.11
Kt/V	−0.01[b]	−0.02
BMI	−0.07	−0.06
Serum calcium	−0.07	−0.01[c]
Serum phosphorous	−0.01[b]	−0.07
Serum iPTH	0.26	0.28
AST	0.27	0.26
Serum albumin	−0.19	−0.11
Serum TIBC	−0.08	−0.01[c]
Serum ferritin	0.09	0.04
Serum bicarbonate	−0.04	−0.05
Serum creatinine	−0.08	−0.10
Blood hemoglobin	−0.09	0.01[d]
WBC count	0.05	0.04
Blood lymphocytes (%)	−0.05	−0.01

[a]$P < 0.001$ except where noted.
[b]$P \geq 0.001$ and ≤ 0.01.
[c]$P \geq 0.05$.
[d]$P \geq 0.01$ and ≤ 0.05.
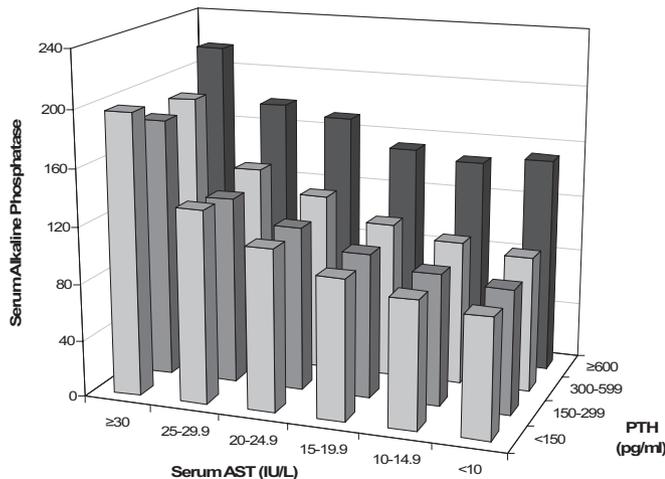
Figure 1. Serum AlkPhos concentrations in the selected strata of the entire spectrum of serum iPTH and AST.

incrementally associated with increased death risk over 3 yr, even after adjustment for surrogates of nutrition, inflammation, minerals, serum PTH, and the liver enzyme AST. This strictly incremental and somewhat monotonous association was observed for both all-cause and cardiovascular mortality and was robust to the method of the survival analyses. These AlkPhos–death associations persist across distinct and mutually exclusive strata of serum PTH and AST and were independent of HCV serology. The mortality predictability of AlkPhos ≥120 U/L was consistent across virtually all subgroups of MHD patients. A rise in serum AlkPhos of >10 U/L during the first 6 mo was associated with the increased death risk in the subsequent 2.5 yr. Deciles of AlkPhos had a more linear association with mortality than deciles of iPTH, which showed a

U-shaped association. These data suggest that in MHD patients, increased serum AlkPhos has a robust and consistent association with mortality.

AlkPhos is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules; this process is also known as dephosphorylation. As the name implies, AlkPhos enzymes are most effective in an alkaline environment. In humans, AlkPhos is present in all solid tissues throughout the entire body but is particularly concentrated in bone, liver and bile duct system, placenta, leukocytes, and kidneys. The usual serum AlkPhos range in healthy individuals is 30 to 100 U/L, although levels up to 120 U/L are usually considered normal in most laboratory centers.[12] Elevated serum AlkPhos levels (hyperphosphatasemia) in adults are often observed in bone disease states characterized by high bone turnover, including high-turnover bone disease and vitamin D deficiency, as well as in obstructive liver and bile duct diseases.[12] Furthermore, bone-specific AlkPhos is a by-product of osteoblasts and is a more specific measure of bone formation as well as bone turnover.[13]

Serum AlkPhos concentration is usually increased in renal osteodystrophy, especially in high-turnover bone disease.[6,8,14] The KDOQI guidelines state that the deleterious effects of high serum PTH levels may be manifested by elevated bone AlkPhos activity as a result of associated bone resorption.[5] The magnitude of the enzyme elevation may indeed be a more reliable marker of severity of the high-turnover osteodystrophy than increased PTH levels, especially because the circulating serum AlkPhos originates directly from the pathologic bone system. Consistent with the foregoing notion, a recent meta-analysis showed that the treatment of renal osteodystrophy by means of vitamin D analogs can effectively decrease AlkPhos, even though such a treatment may not decrease serum PTH consistently,[10] hence the reported link between vitamin D analogs and improved survival in CKD may be via the AlkPhos pathway.[2,15–17]

It is likely that the AlkPhos–mortality association in CKD, including the observed link with cardiovascular death (Figure 2B), is related to vascular calcification through its pyrophosphate link,[18–20] where AlkPhos seems to play a mediating and instrumental role.[21,22] AlkPhos has been shown in histologic sections of vessels obtained from patients with CKD-associated calcific uremic arteriolopathy.[23] Indeed, genetic ablation of tissue-nonspecific AlkPhos leads to amelioration of soft tissue calcification in animal studies.[21] Some novel inhibitors of the physiologic pyrophosphatase activity of AlkPhos are capable of reducing vascular calcification in animal models[21]; however, the AlkPhos–death link may have additional causes, such as its relationship with inflammation or malignancies. A recent study of patients with metastatic neuroendocrine tumors showed that increased AlkPhos levels were predictive of shorter survival in these cancer patients.[24] Moreover, elevated AlkPhos levels may indicate osteomalacia or mineralization deficiency as a result of nutritional 25-hydroxyvitamin D deficiency.[25] The latter may be an independent risk factor for inflammation and cardiovascular disease.[26]

Table 3. Categories of 3-mo averaged AlkPhos and death rates in 73,960 MHD patients

AlkPhos Category (U/L)	n (%)[a]	All-Cause Death (%)[b]	Cardiovascular Death (%)[b]	iPTH (pg/ml)	Calcium (mg/dl)	Phosphorus (mg/dl)	AST (U/L)
<60	6627 (9)	1480 (22)	599 (9)	179 ± 95	9.4 ± 0.7	5.9 ± 1.4	14.3 ± 3.4
60 to 80	16,225 (22)	4115 (25)	1688 (10)	198 ± 105	9.3 ± 0.7	5.8 ± 1.5	15.0 ± 3.5
80 to 100	16,818 (23)	4774 (28)	1914 (11)	217 ± 118	9.3 ± 0.7	5.7 ± 1.5	15.7 ± 3.7
100 to 120	11,697 (16)	3534 (30)	1343 (11)	235 ± 136	9.2 ± 0.7	5.6 ± 1.5	16.3 ± 4.2
120 to 140	7236 (10)	2319 (32)	940 (13)	251 ± 152	9.2 ± 0.7	5.6 ± 1.5	17.0 ± 4.5
140 to 160	4516 (6)	1597 (36)	619 (14)	265 ± 176	9.2 ± 0.8	5.6 ± 1.6	18.0 ± 5.0
160 to 180	2777 (4)	1019 (37)	406 (15)	284 ± 209	9.2 ± 0.8	5.6 ± 1.6	19.0 ± 5.8
≥180	8064 (11)	3275 (41)	1164 (14)	305 ± 285	9.1 ± 0.8	5.7 ± 1.7	22.0 ± 8.5

[a]Values in parentheses represent the proportion of the MHD patients in each AlkPhos category.
[b]Values in parentheses indicate the crude death rates in the indicated group during the 3 yr of observation.
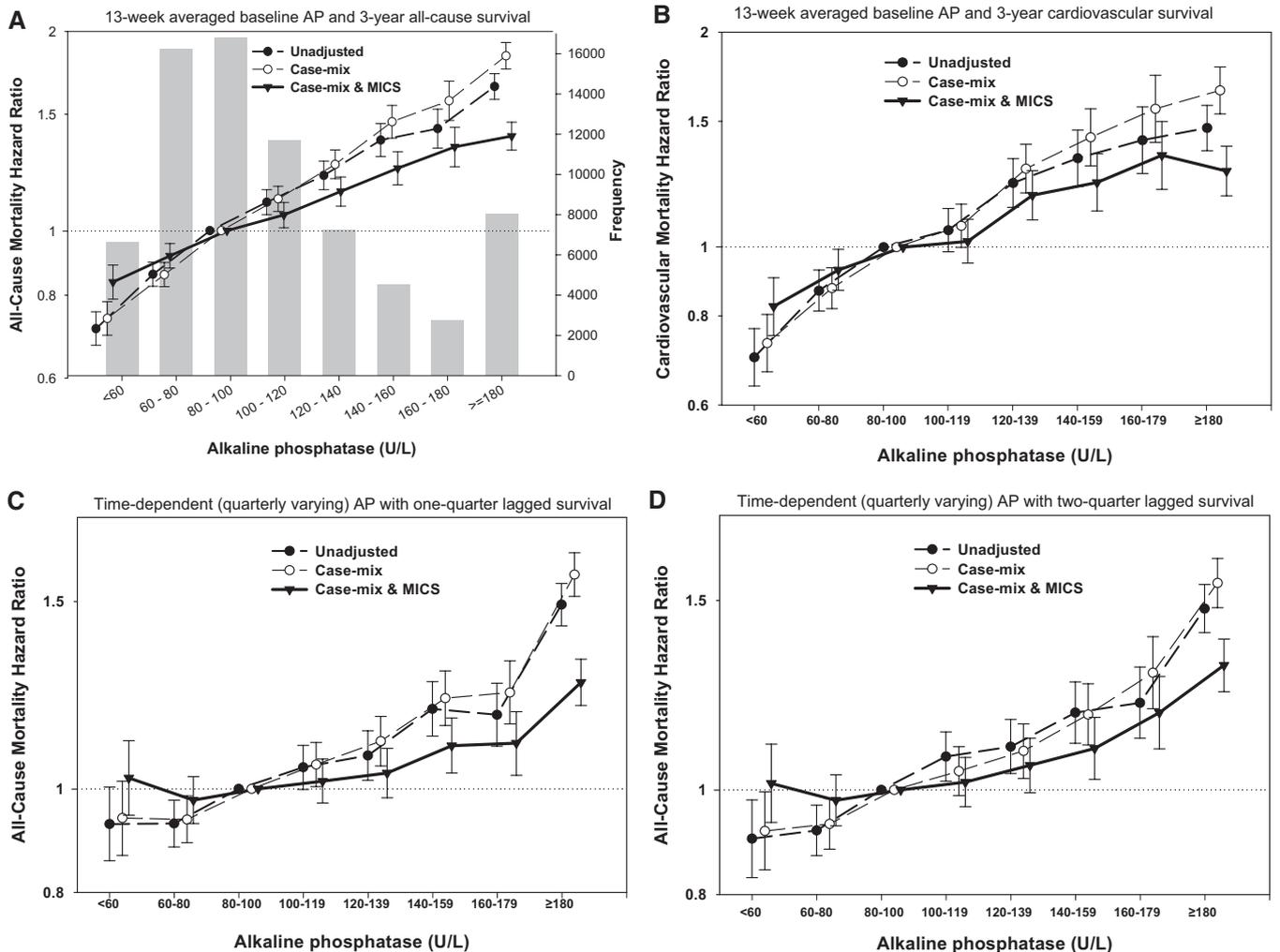
Figure 2. Death hazard ratio for the entire range of serum AlkPhos (AP) in 73,960 MHD patients over 3 yr (July 2001 through June 2004). (A) All-cause mortality with fixed (baseline) covariate model. (B) Cardiovascular mortality with fixed (baseline) covariate model. (C) All-cause mortality with time-varying (calendar quarter) covariate model with one-quarter time lag. (D) All-cause mortality with time-varying (calendar quarter) covariate model with two-quarter time lag. Note that the fully (case mix and MICS) adjusted model also includes adjustment for serum minerals (calcium and phosphorus) and iPTH and AST concentrations. Hazard ratios in y axis are in logarithmic scale.

It is important to note that in time-dependent models (Figure 2, bottom) there was almost no association between AlkPhos values <120 U/L and survival. The observed flat lines were also consistent with the lack of association between a drop in AlkPhos and survival illustrated in Figure 3. The lack of survival improvement trend of lower AlkPhos levels may be

Table 4. All-cause death HR for serum AlkPhos based on time-dependent (quarterly varying) Cox regression model with one calendar quarter lag[a]

AlkPhos Group (U/L)	Unadjusted		Case-Mix Adjusted		Case-Mix and MICS Adjusted	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
<60	0.88 (0.82 to 0.95)	0.002	0.92 (0.85 to 0.99)	0.030	1.03 (0.96 to 1.12)	0.400
60 to 80	0.91 (0.86 to 0.95)	<0.001	0.94 (0.90 to 0.99)	0.010	1.00 (0.96 to 1.05)	0.900
80 to 100 (reference)	1.00	NA	1.00	NA	1.00	NA
100 to 120	1.07 (1.02 to 1.12)	0.003	1.08 (1.03 to 1.13)	<0.001	1.02 (0.97 to 1.06)	0.500
120 to 140	1.12 (1.06 to 1.17)	<0.001	1.16 (1.11 to 1.22)	<0.001	1.04 (0.99 to 1.09)	0.120
140 to 160	1.23 (1.17 to 1.30)	<0.001	1.27 (1.20 to 1.34)	<0.001	1.09 (1.04 to 1.16)	0.001
160 to 180	1.18 (1.11 to 1.26)	<0.001	1.24 (1.16 to 1.32)	<0.001	1.08 (1.02 to 1.16)	0.010
≥180	1.59 (1.53 to 1.66)	<0.001	1.63 (1.56 to 1.70)	<0.001	1.18 (1.13 to 1.24)	<0.001

[a]See also Figure 2C. HR, hazard ratio; NA, not applicable.

Table 5. Categories of *change* in serum AlkPhos during the first 6 mo of the cohort and subsequent death rates in 26,975 MHD patients who had two consecutive averaged AlkPhos values over two calendar quarters and whose baseline serum AlkPhos was between 50 and 200 U/L

| Change Direction	AlkPhos Change over 6 mo (U/L)	n (%)[a]	All-Cause Death (%)[b]	iPTH (pg/ml)	Calcium (mg/dl)	Phosphorus (mg/dl)	AST (U/L)
Decreased	≤−30.0	1229 (5)	475 (39)	377 ± 222	9.2 ± 0.8	5.6 ± 1.4	19.4 ± 15.3
	−29.9 to −20.0	1344 (5)	483 (36)	313 ± 176	9.2 ± 0.7	5.7 ± 1.4	18.1 ± 11.8
	−19.9 to −10.0	2962 (11)	1056 (36)	266 ± 145	9.3 ± 0.7	5.7 ± 1.4	16.7 ± 10.0
Unchanged	−10.0 to 10.0	13,309 (49)	4587 (34)	217 ± 124	9.3 ± 0.7	5.8 ± 1.5	15.7 ± 8.0
Increased	10.0 to 19.9	3740 (14)	1323 (35)	213 ± 127	9.3 ± 0.7	5.8 ± 1.6	16.4 ± 8.3
	20.0 to 29.9	1933 (7)	747 (39)	218 ± 140	9.3 ± 0.7	5.8 ± 1.6	17.3 ± 9.2
	≥30.0	2458 (9)	1120 (46)	236 ± 164	9.3 ± 0.8	5.9 ± 1.7	18.7 ± 17.6

[a]Values in parentheses represent the proportion of the MHD patients in each AlkPhos category.
[b]Values in parentheses indicate the crude death rates in the indicated group during the 3 yr of observation.

related to the higher likelihood of adynamic bone disease in such low ranges.[27–30] Hence, a low AlkPhos can herald adynamic bone, which may *per se* be associated with greater vascular calcification, thereby counteracting the expectedly improving effect of lowering serum AlkPhos. Such a hypothesis, even though biologically plausible and consistent with our discrepant findings pertaining to lower levels of AlkPhos, needs to be verified in future studies.

In our study, the association between serum AlkPhos and survival was monotonic, almost strictly upgoing and independent of the level of multivariate adjustment and irrespective of PTH or AST strata (Figures 2, 5, and 6). This is in sharp contradistinction to the associations between serum mineral or PTH levels and survival or CKD progression, which are usually U- or J-shaped.[1,2,31] A linear association, indicative of the relation between the worsening severity of high-turnover bone disease and increased risk for death, more optimally fulfills the "dose-response criterion," which is one of the nine principles of the "Hill's Causality Criteria."[26,32,33] Another Hill's criterion, the "temporal relationship," indicates that the exposure should precede the outcome.[26,32,33] This was also observed in this study. Nevertheless, as is the case with all observational research, associative data suggest but cannot ensure causality.

A limitation of this study is that the AlkPhos measured routinely in dialysis patients is not bone specific. Liver disease may be associated with increased serum AlkPhos level. Indeed, we found a moderate correlation ($r = 0.26$ to 0.27) between AlkPhos and the liver enzyme AST in our study; however, even though liver diseases may be associated with increased liver enzyme and with increased death risk in MHD patients,[34] a large proportion of the mortality predictability of AlkPhos is likely due to renal osteodystrophy, especially because patients with a high AST >30 U/L composed only <10% of our studied cohort. Moreover, the case-mix and case-mix plus malnutrition-inflammation complex syndrome (MICS)-adjusted analyses were adjusted for serum AST levels, an indicator of hepatic disease. Use of bone-specific AlkPhos and/or controlling for additional liver enzymes or other surrogates of liver disease, such as hepatitis C, could have better delineated the liver independence of the AlkPhos–death association in MHD patients[9,35–37]; however, bone AlkPhos or other liver enzymes are not measured routinely in American MHD patients, whereas total AlkPhos and AST are measured monthly in virtually everybody. Furthermore, the commercially available bone AlkPhos immunoassays seem unable to distinguish the AlkPhos isoform–specific differences optimally.[6,38,39] In a recent study of MHD patients with renal osteodystrophy, the presence or
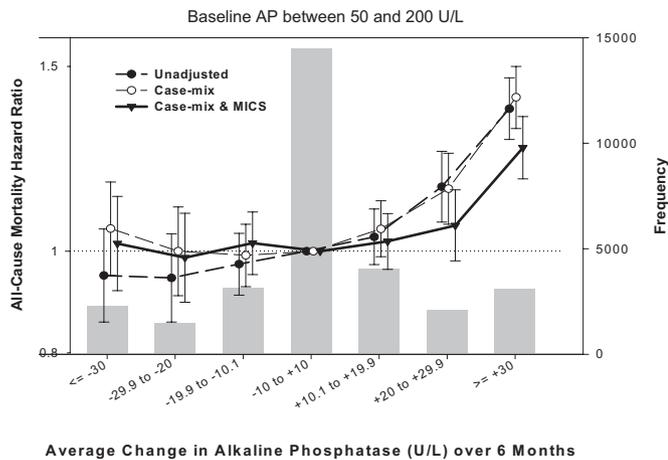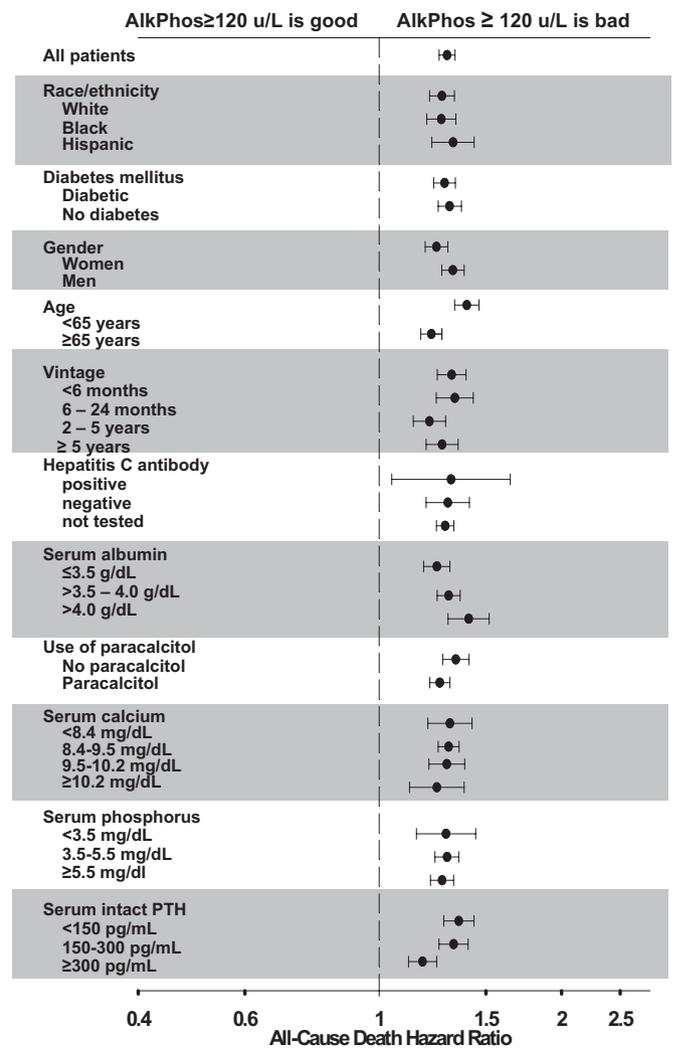
Figure 3. Hazard ratio of all-cause mortality for the changes in serum AP from the baseline to subsequent calendar quarter (*i.e.*, over 6 mo in 26,975 MHD patients), with subsequent survival over the subsequent 2.5 yr. All models are also adjusted for baseline AP. Note that the fully (case mix and MICS) adjusted model also includes adjustment for serum minerals (calcium and phosphorus) and iPTH and AST concentrations. Hazard ratios in *y* axis are in logarithmic scale.

absence of anti–hepatitis C antibodies did not affect the relationship between the biochemical markers of bone metabolism and PTH levels.[40]

Our study should be qualified for its observational-epidemiologic nature, its retrospective nature, and 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 (TIBC), white blood cell count, lymphocyte percentage, hemoglobin, and administered erythropoietin dosage, which have significant associations with inflammation in MHD patients.[41,42] Another limitation of our analysis is that it is based on a 3-yr period of the cohort, rather than a longitudinal follow-up of many years. Nonetheless, almost half of dialysis patients in the United States are usually dead within 3 yr of commencing MHD treatment; therefore, any insight into the short-term survival of dialysis patients is of major clinical relevance. The strengths of our study include (*1*) its contemporary nature, because all patient data were obtained from the 21st century (2001 through 2004); (*2*) uniform laboratory measurements with all laboratory data obtained from one single facility; (*3*) large sample size; (*4*) 3-mo averaged laboratory data, in that most values are the means of several measurements to minimize measurement variability; and (*5*) additional use of time-dependent survival models and sensitivity analyses.

In conclusion, we showed that increased serum AlkPhos is incrementally associated with increased mortality independent of other markers of CKD-MBD, including serum minerals and PTH and independent of the liver enzyme AST. This AlkPhos–death association is robust and holds in different subgroups of MHD patients. Because high-turnover bone disease, manifested by high serum AlkPhos levels,
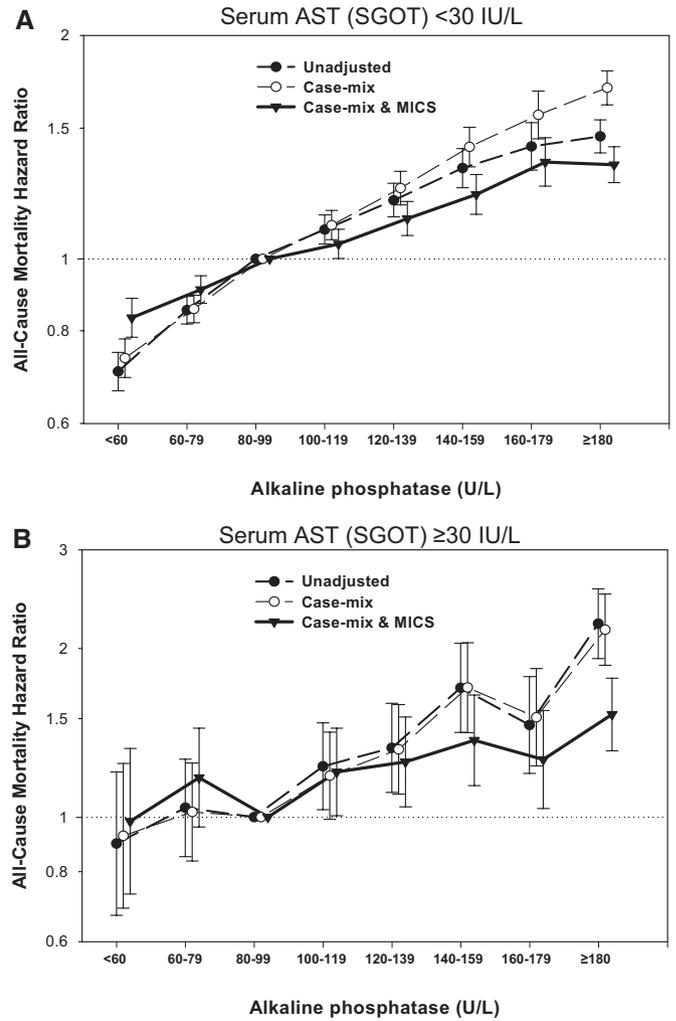

Figure 4. Hazard ratio of all-cause mortality for the dichotomized AP ≥120 U/L at baseline in various subgroups of 73,960 MHD patients over 3 yr. Note that the model is adjusted for all case mix and MICS markers, including serum minerals (calcium and phosphorus) and iPTH and AST concentrations. Note that hepatitis C antibody data were available only for 18% of the cohort (*n* = 13,664). Hazard ratios in *y* axis are in logarithmic scale.

among others, is common in the CKD population and can be effectively treated with vitamin D analogs[26] or calcimimetics[43] and because serum PTH measurements in CKD may be confounded by such conditions as obesity[7] or suboptimal assays,[44–46] our findings may have important clinical implications on renewing attention to serum AlkPhos measurement and evaluation.[47] Although PTH and AlkPhos are frequently in agreement, there may be subtle differences leading to major differences in clinical outcomes consistent with the discrepant findings shown in Figure 7; it seems important to separate parathyroid activity signaled by PTH from bone remodeling reflected by AlkPhos. Even though we found that a serum AlkPhos >120 U/L is associated with increased death risk in all groups of MHD patients, KDOQI
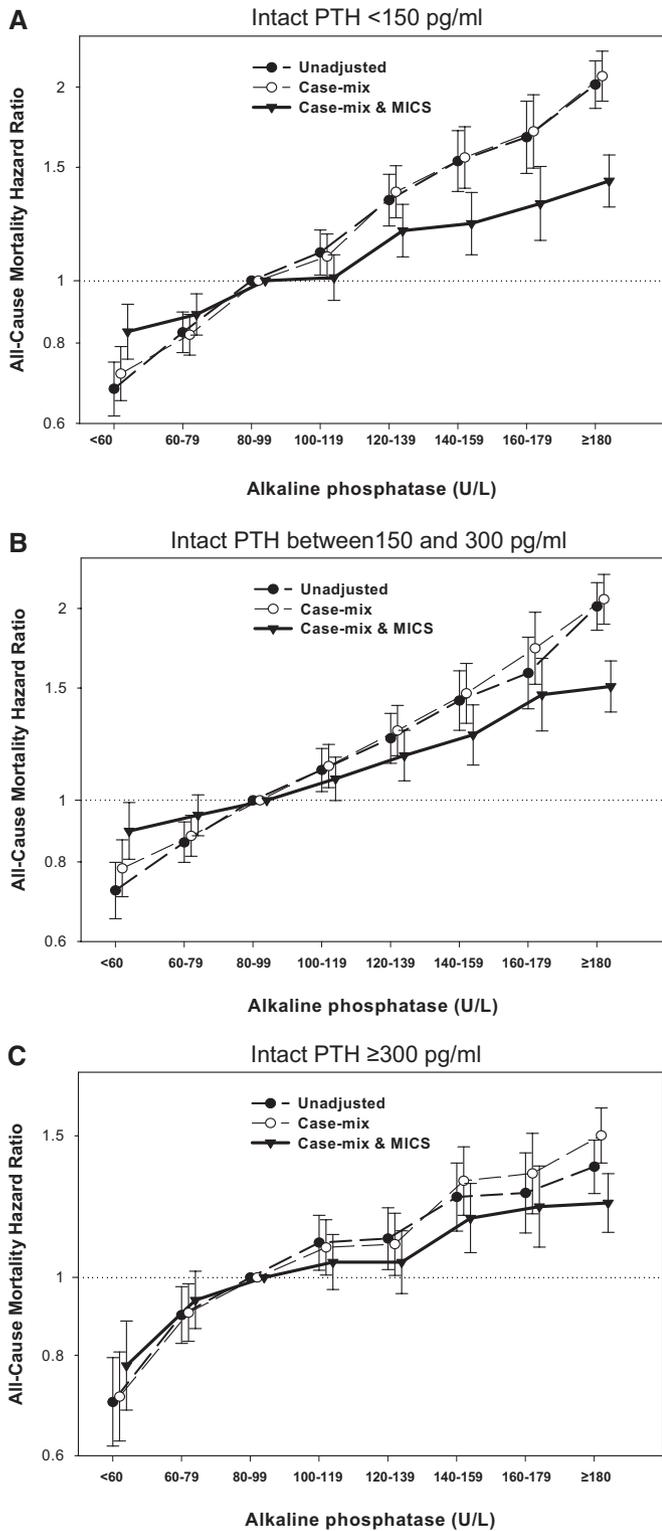
Figure 5. Hazard ratio of all-cause mortality for AlkPhos at baseline in three strata of serum iPTH in 73,960 MHD patients over 3 yr. (A) Low iPTH <150 pg/ml (n = 20,775). (B) K/DOQI recommended target zone for iPTH (between 150 and 300 pg/ml; n = 22,543). (C) High iPTH ≥300 pg/ml (n = 23,912). Note that the fully (case mix and MICS) adjusted model also includes adjustment for serum minerals (calcium and phosphorus) and iPTH and AST concentrations. Hazard ratios in y axis are in logarithmic scale.

Figure 6. Hazard ratio of all-cause mortality for AlkPhos at baseline in two strata of serum AST in 73,960 MHD patients over 3 yr. (A) Low to normal AST <30 IU/L (n = 66,868). (B) High AST ≥ 30 IU/L (n = 6708). Note that the fully (case mix and MICS) adjusted model also includes adjustment for serum minerals (calcium and phosphorus) and iPTH and AST concentrations. Hazard ratios in y axis are in logarithmic scale.

has not yet recommended any specific target zone as it has done for PTH. Because of the significant association of osteodystrophy with cardiovascular calcification, cardiovascular disease, and death, diligent treatment of high-turnover bone disease may be an effective measure to improve survival in CKD. Close monitoring of AlkPhos levels may be useful when considering initiation or changes of the therapy. Prospective controlled trials are needed to verify the true relationships between serum AlkPhos and outcome in MHD patients; to better understand the natural course of renal osteodystrophy and its complications in CKD; and to evaluate the effectiveness of current and future treatments including vitamin D analogs, calcimimetics, and other medications such as AlkPhos inhibitors in improving osteodystrophy and clinical outcome in CKD population.
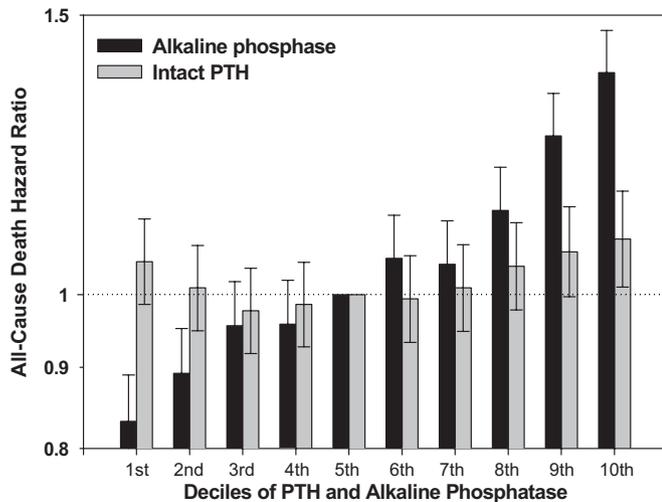
Figure 7. Comparing mortality predictability of deciles of serum AlkPhos and deciles of iPTH in 80,000 MHD patients who were followed for up to 3 yr (2001 through 2004). Note that these analyses are based on fixed covariate (non–time dependent) and fully adjusted models. The fifth decile serves as the reference group. The cutoff levels for AlkPhos deciles are 61, 71, 79, 87, 96, 107, 121, 142, and 186 U/L and for iPTH are 64, 106, 146, 184, 224, 274, 344, 459, and 697 pg/ml. Each decile includes approximately 7400 MHD patients. Hazard ratios in y axis are in logarithmic scale.

CONCISE METHODS

Patients

We extracted, refined, and examined data from all individuals who had stage 5 CKD and underwent MHD treatment from July 2001 to June 2004 in one of the 580 outpatient dialysis facilities of a large dialysis organization in the United States (DaVita, Inc., before its acquisition of former Gambro dialysis facilities). The study was approved by the institutional review committees of both Los Angeles Biomedical Research Institute at Harbor-UCLA and DaVita Clinical Research. Because of the large sample size studied, the anonymity of the patients studied, and the nonintrusive nature of the research, the requirement for a written consent form was exempted.

Clinical and Demographic Measures

The creation of the 3-yr cohort has been described previously.[11,48–50] For minimization of measurement variability, all repeated measures for each patient during any given calendar quarter (*i.e.*, during a 13-wk interval) were averaged and the summary estimate was used in all models. Averaged values were obtained for up to 12 calendar quarters (Q1 through Q12) for each laboratory and clinical measure for each patient during the 3-yr cohort period. Dialysis vintage was defined as the duration of time between the first day of dialysis treatment and the first day that the patient entered the cohort. The first (baseline) studied quarter for each patient was the calendar quarter in which patient's vintage was >90 d during at least half of the time of that given quarter.

Thirteen-week averaged postdialysis weight and baseline height were used to calculate the body mass index (BMI; weight [kg]/height

squared [m²]). The dosages of injected recombinant human erythropoietin (Epogen; Amgen, Inc., Thousand Oaks, CA), 1,25-$(OH)_2D_3$ (calcitriol; Calcijex; Abbott Laboratories, Abbott Park, IL), and 19-Nor-1,25-$(OH)_2D_2$ (paricalcitol; Zemplar; Abbott Laboratories) were also calculated for each calendar quarter. Computerized causes of death were obtained, and cardiovascular death was defined as death as a result of myocardial infarction, cardiac arrest, heart failure, cerebrovascular accident, and other cardiac causes.

In addition to the presence or absence of diabetes, which was available in the database, histories of tobacco smoking and preexisting comorbid conditions were obtained by linking the DaVita database to the Medical Evidence Form 2728 of the US Renal Data System[51] and categorized into 11 comorbid conditions: (*1*) Ischemic heart disease, (*2*) congestive heart failure, (*3*) status post (s/p) cardiac arrest, (*4*) s/p myocardial infarction, (*5*) pericarditis, (*6*) cardiac dysrhythmia, (*7*) peripheral vascular disease, (*8*) chronic obstructive pulmonary disease, (*9*) HIV/AIDS status, (*10*) ambulatory status, and (*11*) cancer.

Laboratory Measures

Blood samples were drawn using uniform techniques in all of the DaVita dialysis clinics and were transported to the DaVita Laboratory (Deland, FL) typically within 24 h. All laboratory values were measured by automated and standardized methods in the DaVita Laboratory. Roche modular instrumentation method[52] (Roche Diagnostics Corp., Indianapolis, IN) was used for quantitative determinations of AlkPhos in that p-nitrophenyl phosphate is converted to p-nitrophenol plus phosphate, where p-nitrophenol released is proportional to the AlkPhos activity and is measured photometrically. Measured imprecision studies using DaVita patient samples recovered a coefficient of variation of <2.0% and an extended reportable range of 1.0 to 4400 U/L (Dr. J. Steinmetz, DaVita Laboratories, personal communication, March 25, 2008).

Most laboratory values, including complete blood cell counts and serum levels of AlkPhos, AST, urea nitrogen, creatinine, albumin, calcium, phosphorus, bicarbonate, and TIBC, were measured monthly. Serum ferritin and iPTH were measured at least quarterly. Hemoglobin was measured at least monthly in essentially all patients and weekly to biweekly in most patients. Kt/V was used to estimate dialysis dosage, and normalized protein equivalent of total nitrogen appearance, also known as normalized protein catabolic rate, an estimation of daily protein intake, was measured monthly as a measure of protein intake. The HCV antibody status was examined using the third generation of the HCV enzyme immunoassay (EIA version 2.0; Abbott Laboratories).[11] Most blood samples were collected before dialysis, with the exception of the postdialysis serum urea nitrogen that was obtained to calculate urea kinetics.

Epidemiologic and Statistical Methods

Survival analyses included Cox proportional hazards regression modeling with either baseline values and fixed covariates or with time-dependent (quarterly varying) values using repeated measures that were averaged over each 13-wk calendar quarter (season). In time-dependent Cox models, we examined the association between quarterly averaged serum AlkPhos and the subsequent survival with a time

lag of one quarter and two quarters separately. For each analysis, three models were examined on the basis of the level of multivariate adjustment:

1. An unadjusted model that included mortality data, AlkPhos categories, and entry calendar quarter (Q1 through Q12)

2. Case mix–adjusted models that included all of the above plus age, gender, race and ethnicity (African American and other self-categorized black, non-Hispanic white, Asian, Hispanic, and other), diabetes and 11 preexisting comorbid states, history of tobacco smoking, categories of dialysis vintage (<6 mo, 6 mo to 2 yr, 2 to 5 yr, and ≥5 yr), primary insurance (Medicare, Medicaid, private, and others), marital status (married, single, divorced, widowed, and other or unknown), the standardized mortality ratio of the dialysis clinic during entry quarter, dialysis dosage as indicated by Kt/V (single pool), presence or absence of a dialysis catheter, and residual renal function during the entry quarter (*i.e.*, urinary urea clearance, and AST as a marker of liver function)

3. MICS-adjusted models, which included all of the covariates in the case-mix model as well as 13 surrogates of nutritional status and inflammation, including BMI, the average dosage of paracalcitol or calcitriol (no other injectable vitamin D analog was administered), and 12 laboratory variables as surrogates of the nutritional state or inflammation or minerals with known association with clinical outcomes in MHD patients: (*1*) Normalized protein equivalent of total nitrogen appearance as an indicator of daily protein intake, (*2*) serum albumin, (*3*) serum TIBC, (*4*) serum ferritin, (*5*) serum creatinine, (*6*) serum phosphorus, (*7*) serum calcium, (*8*) iPTH, (*9*) serum bicarbonate, (*10*) peripheral white blood cell count, (*11*) lymphocyte percentage, and (*12*) hemoglobin.

Patients who received a transplant, switched to peritoneal dialysis, or left DaVita clinics were censored at the time of the event. Missing covariate data (<2% for most laboratory and demographic variables and <18% for any of the 10 comorbid conditions) were imputed by the mean or median of the existing values, whichever was most appropriate. All descriptive and multivariate statistics were carried out with SAS 9.1 (SAS Institute, Cary, NC) and Stata 9.0 (Stata Corp., College Station, TX).

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DISCLOSURES

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