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Prognostic associations of serum calcium, phosphate and calcium phosphate concentration product with outcomes in kidney transplant recipients

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Summary

Disturbances in calcium and phosphate metabolism have been linked to increased mortality in hemodialysis patients, but not in kidney transplant recipients (KTR). We enrolled 733 KTR from the Vienna General Hospital into this study. Detailed demographic, clinical, laboratory, and transplant-related information was collected at baseline. We used the Austrian Dialysis and Transplantation Registry for follow-up. Using multivariate proportional hazard regression, we examined the independent associations between serum calcium, serum phosphate, and calcium phosphate ($CaPO_4$) product with the outcomes of death from any cause and kidney allograft loss. Over a median follow-up of >6 years, 154 patients died and 259 kidney allografts were lost. Associations with serum calcium, phosphate concentrations, and $CaPO_4$ product concentrations were found for allograft loss, but not for patient mortality. Patients in the highest quintile of phosphate concentration and $CaPO_4$ product had an increased risk for allograft loss compared with patients in the lowest quintile of these parameters (hazards ratio, HR = 2.15; 95% confidence interval, CI: 1.36–3.40 and HR = 1.72; 95% CI: 1.10–2.71, respectively). High calcium levels were associated with a reduced risk for allograft loss. Results were even more pronounced for death-censored allograft loss. High concentrations of serum phosphate and $CaPO_4$ product were associated with an increased risk for allograft loss in these KTR, whereas high serum calcium concentrations seemed to lower the risk.

Introduction

Whereas improved immunosuppressive regimens have increased the median patient and graft survival time during the past decades by decreasing the acute failure rate, chronic allograft nephropathy in many cases is still an unsolved problem and the dominant cause of transplant failure [1]. At the time of transplantation, immunologic as well as non-immunologic factors have been studied abundantly. However, amazingly little attention has been paid to abnormalities in the mineral metabolism, which are very common in patients with chronic kidney disease (CKD) and on hemodialysis. This abnormal mineral metabolism continues in a significant number of kidney transplant recipients (KTR).

Hyperphosphatemia, hypocalcemia, as well as hypercalcemia, and hyperparathyroidism are common findings in patients with end-stage renal disease (ESRD),

especially in patients who comply poorly with dietary phosphate restriction or prescribed supplemental phosphate binders. It has been shown that elevated serum phosphate concentrations as well as an elevated calcium-phosphate ($CaPO_4$) concentration product are associated with an increased risk for mortality in hemodialysis patients [2]. Successful renal transplantation, which may lead to normalization of urinary phosphate excretion and renal calcitriol production may reverse hyperparathyroidism because of involution of the parathyroid glands [3]. This process, however, appears to take a few months to several years, thus resulting in many KTR presenting with persistently elevated serum calcium concentrations. Hyperplastic parathyroid glands may involute slowly despite persistant hypercalcemia; severely enlarged glands may never return to normal function or size. Bearing in mind that an elevated serum $CaPO_4$ concentration product is associated with an increased cardiovascular risk in hemodialysis and CKD patients and that an elevated parathyroid hormone is an independent predictor for left ventricular hypertrophy [4], KTR may also be at greater risk for vascular calcifications and mortality.

Hypophosphatemia as a consequence of persisting hyperparathyroidism is also a well-recognized problem during the first weeks after transplantation [5]. This problem seems to be aggravated by rapamycin-based immunosuppression [6], and often persists for several months after transplantation even if the renal function is stable [7]. Apart from the well-described musculoskeletal complications of hypophosphatemia, data on the impact of markedly decreased phosphate on mortality or allograft loss are missing.

As the prognostic significance of calcium, phosphate concentrations and their product regarding long-term patient and allograft survival have not sufficiently been established in KTR, we undertook this prospective study. We sought to test whether serum calcium, serum phosphate levels and $CaPO_4$ concentration product were associated with all-cause mortality and the risk of kidney allograft loss in KTR.

Patients and methods

Study population and data collection

Between 1996 and 1998, we prospectively enrolled 733 prevalent and stable KTR who received routine follow-up at the transplant clinic of the Vienna General Hospital into this cohort. All patients gave informed consent in accordance with the Declaration of Helsinki. A detailed description of this cohort has been reported previously [8]. All clinical characteristics were assessed at study baseline; during the baseline visit, blood was also drawn from each patient for laboratory analysis.

Study outcomes

The outcomes of this study were all-cause mortality and kidney allograft loss as well as death-censored kidney allograft loss; kidney allograft loss was defined as the composite end point of patient death and re-initiation of maintenance dialysis. Additional analyses using graft failure as the main outcome but using death as a censoring rather than an outcome event were also conducted.

Main exposures

At baseline, we measured each participant's serum calcium and serum phosphate concentrations (in mmol/l). From these, we calculated the product of these measurements, the $CaPO_4$ product (in $mmol^2/l^2$). Patients were categorized into quintiles of these exposure variables.

Covariates

We assessed each patient's age at baseline, gender, underlying renal disease, number of previous kidney transplants, time from first renal replacement therapy to transplantation, and time since the most recent kidney transplantation. Body mass index was calculated as the weight in kilograms divided by the squared height in meters. The immunosuppressive regimen at study baseline was also noted. We also measured each patient's C-reactive protein (CRP), total fasting homocysteine, and creatinine concentrations. We then used the Cockcroft–Gault formula to estimate creatinine clearance, a proxy for glomerular filtration rate, which was standardized to a body surface area of 1.73 m^2 [9].

From the Eurotransplant Foundation's organ procurement registry, we obtained information on the organ donor (donor age, gender, and living versus deceased donor), and on the specific circumstances of the transplantation procedure (cold ischemia time, number and type of human leukocyte antigen mismatch, and recipient panel reactive antibody titer). All patients' baseline records were linked to the data from the Austrian Dialysis and Transplant Registry (OeDTR). Follow-up information on all dialysis patients and KTR residing in Austria is routinely collected by the Austrian Society of Nephrology and has been practically complete for many years. Thus, reliable information on timing and occurrence of patient death and modality switches, such as re-initiation of maintenance dialysis after kidney graft failure, is available for study.

Statistical analyses

All analyses used the SAS for Windows software, version 9.1 (The SAS Institute, Cary, NC). Patients were followed from the date of study inclusion until they reached an end-point or to the date when they were last seen at the transplant clinic. Baseline characteristics were compared using *t*-test for continuous variables and Pearson's chi-squared test for categorical variables. We used Kaplan–Meier survival curves to describe the cumulative incidence of all-cause mortality and kidney allograft loss over time, and the log-rank test was applied to test for differences among the quintiles of baseline serum phosphate and serum calcium concentrations. Univariate and multivariate Cox proportional hazards models were then fit to test for association between these putative risk factors and the study outcomes. Age, gender, and eGFR were forced into all multivariate models. Full multivariate models were then selected using an automated stepwise selection algorithm available in SAS (at $P < 20$). We then introduced each variable that had not automatically been selected and assessed whether its introduction changed any of the effect estimates of the study exposure variables by >10%, which would be regarded an indication for residual confounding by that variable. No variable satisfied this criterion in any of the outcomes models considered. We estimated univariate and multivariate hazards ratios (HR) and the corresponding 95% confidence intervals (CI). Associations between continuous covariates and the outcomes were examined for linearity and used in categories otherwise. Body mass index was used in five categories, as its *U*-shaped association with the study outcomes has been well-established [10].

Results

Seven of the 733 patients originally included in the study had to be excluded, as they were found to receive their routine transplant care outside Austria. For 16 patients, information on their donors was unavailable from the Eurotransplant Foundation registry, and two patients had calcium concentration measurements that were biologically implausible. The remaining 708 patients constituted the final study cohort. On average, patients had received their current transplant 5 years before study enrollment. The mean recipient age was 52 years, and 60% were men (Table 1). Patients had a mean body mass index of 25 kg/m^2 and the mean estimated creatinine clearance was 56 ml/min/1.73 m^2. The mean serum concentration of calcium was 2.37 mmol/l, 1.04 mmol/l for serum phosphate, and 2.46 mmol^2/l^2 for the $CaPO_4$ concentration product. Other baseline characteristics are displayed in Table 1, and specific characteristics regarding the transplantation procedures are available in Table 2.

Table 1. Baseline Characteristics of 708 kidney transplant recipients.

Variable	*N* (%) or mean (±SD)
Recipient age (years)	52.2 (±13.3)
Recipient gender (male)	427 (60.1)
Glomerular filtration rate (ml/min/1.73 m^2)	55.8 (±20.0)
Body mass index (kg/m^2)	25.4 (±4.3)
Hemoglobin (g/dl)	12.8 (±2.0)
Calcium (mmol/l)	2.37 (±0.16)
Phophate (mmol/l)	1.04 (±0.27)
Ca × PO_4 (mmol^2/l^2)	2.46 (±0.62)
Total plasma homocysteine (μmol/l)	17.2 (±8.8)
C-reactive protein (mg/dl)	
≤0.5	531 (75.5)
0.5–1.0	86 (12.2)
>1.0	86 (12.2)
Underlying renal disease	
Diabetic nephropathy	47 (6.6)
Glomerulonephritis	243 (34.2)
Interstitial nephritis	114 (16.1)
Polycystic kidney disease	97 (13.7)
Various other, specified	59 (8.3)
Unspecified/unknown	150 (21.1)

SD, standard deviation.
To convert glomerular filtration rate from ml/min/1.73 m^2 to ml/s/1.73 m^2 multiply with 0.01667; to convert hemoglobin from g/dl to g/l multiply with 0.1; to convert calcium from mmol/l to mg/dl multiply with four, to convert phosphate from mmol/l to mg/dl multiply with 3.1; to convert total cholesterol from mg/dl to mmol/l multiply with 0.02586; to convert triglyceride from mg/dl to mmol/l multiply with 0.01129; to convert total homocysteine from μmol/l to mg/l multiply with 7.397.

Endpoint: all-cause mortality

During a median follow-up of 6.1 years (3798 person-years), 154 patient deaths were recorded (crude incidence rate 40.6/1000 person-years). The number of patients at risk and outcomes observed in each exposure quintile are shown in Table 3. From univariate Cox proportional hazard models, we found that people in the highest quintile of baseline serum calcium concentration (≥2.50 mmol/l) had a lower risk for all-cause mortality compared with KTRs in the lowest quintile (Ca ≤2.25 mmol/l; HR = 0.54; 95% CI: 0.32–0.92). After multivariate adjustment, however, this association was attenuated and the confidence interval included the null value of no association (HR = 0.65; 95% CI: 0.38–1.13). No univariate or multivariate associations with all-cause mortality were found across quintiles of serum phosphate concentration or the $CaPO_4$ concentration product (Table 4).

Table 2. Transplantation-related characteristics of 708 kidney transplant recipients.

Variable *N* (%) or mean (±SD)	Total *N* (%)
Immunosuppressive regimen	
Cyclosporin A + Corticosteroid + Azathioprine	317 (44.7)
Cyclosporin A + Corticosteroid	185 (26.1)
Cyclosporin A + Corticosteroid + MMF	126 (17.8)
Other	82 (11.6)
Time from first RRT to transplantation (years)	3.2 (±3.6)
Time since transplantation (years)	5.0 (±4.0)
Number of previous kidney transplants	
0	576 (81.1)
1	113 (15.9)
2/3	21 (3.0)
Donor organ type (living versus deceased)	33 (4.7)
Donor age (years)	38.4 (±15.6)
Donor gender (male)	449 (63.2)
Number of HLA-mismatches	2.2 (±1.2)
Cold ischemia time (hours)	20.8 (±7.7)
Panel reactive antibody titer (>50% vs. ≤50%)	47 (6.9)

SD, standard deviation; HLA, human leucocyte antigen; RRT, renal replacement therapy; MMF, mycophenolate mofetil.

Endpoint: kidney allograft loss

For study of the combined endpoint of allograft loss (alive or associated with death), 3480 person-years of follow-up were available, and this event was observed in 259 patients (crude incidence rate 74.7/1000 person-years). In univariate Cox proportional hazard models, the risk for allograft loss again decreased with increasing calcium concentration revealing statistically significant associations between a calcium concentration of ≥2.33 mmol/l and allograft loss with the lowest HR for the people with a serum calcium ≥2.5 mmol/l (HR = 0.45; 95% CI: 0.30–0.66). After multivariate adjustment these associations were attenuated slightly but remained significant for quintile 3 and 5 (Table 5).

By contrast, we found a direct association between serum phosphate concentrations and allograft loss. Compared with KTRs in the lowest quintile (<0.84 mmol/l), patients with serum phosphate concentrations ≥1.09 mmol/l had an increased risk of allograft loss (HR = 1.56; 95% CI: 1.01–2.42 for phosphate between 1.09 and 1.22 mmol/l; HR = 3.53; 95% CI: 2.37–5.26 for phosphate ≥1.23 mmol/l). Multivariate adjustment attenuated these associations, but serum phosphate concentrations ≥1.23 mmol/l remained independently associated with the risk of allograft loss (HR = 2.15; 95% CI: 1.36–3.40).

Similar results could be observed for the $CaPO_4$ concentration product with statistically significant associations in univariate analysis for an increased risk for concentration products ≥2.57 $mmol^2/l^2$ (HR = 1.73; 95% CI: 1.13–2.63 for $CaPO_4$ 2.57–2.89 $mmol^2/l^2$, and HR = 3.14; 95% CI: 2.11–4.67 for $CaPO_4$ ≥2.90 $mmol^2/l^2$) and weaker but still statistically significant associations for concentrations ≥2.9 $mmol^2/l^2$ after multivariate analysis (HR = 1.72; 95% CI: 1.10–2.71).

Table 3. Number of patients, outcomes, and baseline renal function by quintile of calcium concentration, phosphate concentration and their product.

	Quintile (lowest to highest)				
	1	2	3	4	5
Serum calcium (mmol/l)	≤2.25	2.26–2.32	2.33–2.40	2.41–2.49	≥2.50
Patients	148	130	152	138	141
Deaths	39	30	37	26	22
Allograft losses	72	52	50	43	40
Estimated GFR (ml/min/1.73 m^2)	29.3	57.5	58.1	56.4	58.3
Serum phosphate (mmol/l)	≤0.84	0.85–0.96	0.97–1.08	1.09–1.22	≥1.23
Patients	140	142	148	138	140
Deaths	23	29	37	30	35
Allograft losses	35	48	38	56	80
Estimated GFR (ml/min/1.73 m^2)	64.3	63.2	57.3	52.9	41.2
Calcium phosphate product ($mmol^2/l^2$)	≤2.00	2.00–2.29	2.29–2.57	2.57–2.89	≥2.90
Patients	141	142	142	142	141
Deaths	26	34	27	34	33
Allograft losses	34	39	51	49	84
Estimated GFR (ml/min/1.73 m^2)	64.2	61.5	60.8	51.8	40.1

GFR, glomerular filtration rate.

Table 4. Associations with all-cause mortality of serum calcium concentration, serum phosphate concentration, and calcium phosphate concentration product.

	HR (95% CI)	
	Univariate	Multivariate
Serum calcium concentration (in mmol/l)		
Quintile 1 (≤2.25)	1.0 (referent)	1.0 (referent)
Quintile 2 (2.26–2.32)	0.82 (0.51–1.33)	0.87 (0.54–1.45)
Quintile 3 (2.33–2.40)	0.86 (0.55–1.35)	0.95 (0.60–1.51)
Quintile 4 (2.41–2.49)	0.65 (0.39–1.06)	0.85 (0.50–1.44)
Quintile 5 (≥2.50)	0.54 (0.32–0.92)	0.65 (0.38–1.13)
Serum phosphate concentration (in mmol/l)		
Quintile 1 (≤0.84)	1.0 (referent)	1.0 (referent)
Quintile 2 (0.85–0.96)	1.29 (0.75–2.22)	1.46 (0.83–2.58)
Quintile 3 (0.97–1.08)	1.57 (0.93–2.64)	1.70 (0.99–2.91)
Quintile 4 (1.09–1.22)	1.36 (0.79–2.34)	1.28 (0.72–2.26)
Quintile 5 (≥1.23)	1.57 (0.93–2.66)	1.41 (0.78–2.57)
Serum calcium phosphate product (in $mmol^2/l^2$)		
Quintile 1 (≤2.00)	1.0 (referent)	1.0 (referent)
Quintile 2 (2.00–2.29)	1.36 (0.82–2.27)	1.58 (0.94–2.66)
Quintile 3 (2.29–2.57)	1.04 (0.60–1.77)	1.30 (0.74–2.26)
Quintile 4 (2.57–2.89)	1.32 (0.79–2.21)	1.20 (0.69–2.06)
Quintile 5 (≥2.90)	1.28 (0.77–2.14)	1.10 (0.61–2.00)

Multivariate models additionally adjusted for recipient age, gender, estimated glomerular filtration rate, C-reactive protein, total plasma homocysteine, body mass index, diabetic nephropathy, donor gender, time from first renal replacement therapy to transplantation; multivariate models contain only one calcium/phosphate parameter (in categories) at a time.

Table 5. Associations with kidney allograft loss of serum calcium concentration, serum phosphate concentration, and calcium phosphate concentration product.

	HR (95% CI)	
	Univariate	Multivariate
Serum calcium concentration (in mmol/l)		
Quintile 1 (≤2.25)	1.0 (referent)	1.0 (referent)
Quintile 2 (2.26–2.32)	0.70 (0.49–1.00)	0.89 (0.61–1.29)
Quintile 3 (2.33–2.40)	0.52 (0.37–0.75)	0.68 (0.47–0.99)
Quintile 4 (2.41–2.49)	0.50 (0.34–0.73)	0.75 (0.50–1.12)
Quintile 5 (≥2.50)	0.45 (0.30–0.66)	0.61 (0.40–0.93)
Serum phosphate concentration (in mmol/l)		
Quintile 1 (≤0.84)	1.0 (referent)	1.0 (referent)
Quintile 2 (0.85–0.96)	1.16 (0.74–1.85)	1.30 (0.81–2.09)
Quintile 3 (0.97–1.08)	1.51 (0.98–2.34)	1.41 (0.89–2.23)
Quintile 4 (1.09–1.22)	1.56 (1.01–2.42)	1.34 (0.84–2.12)
Quintile 5 (≥1.23)	3.53 (2.37–5.26)	2.15 (1.36–3.40)
Serum calcium phosphate product (in $mmol^2/l^2$)		
Quintile 1 (≤2.00)	1.0 (referent)	1.0 (referent)
Quintile 2 (2.00–2.29)	1.47 (0.95–2.27)	1.34 (0.86–2.08)
Quintile 3 (2.29–2.57)	1.10 (0.70–1.75)	1.11 (0.69–1.77)
Quintile 4 (2.57–2.89)	1.73 (1.13–2.63)	1.31 (0.84–2.04)
Quintile 5 (≥2.90)	3.14 (2.11–4.67)	1.72 (1.10–2.71)

Multivariate models additionally adjusted for recipient age, gender, estimated glomerular filtration rate, C-reactive protein, total plasma homocysteine, body mass index and diabetic nephropathy, donor age, time from first renal replacement therapy to transplantation; multivariate models contain only one calcium/phosphate parameter (in categories) at a time.

Endpoint: death-censored kidney allograft loss

As kidney allograft loss was defined as the composite end point of patient death and re-initiation of maintenance dialysis we conducted additional analyses of death-censored kidney allograft loss. Table 6 presents decreased risks for death-censored allograft loss for patients in the highest quintile of serum calcium concentration

Table 6. Associations with death-censored kidney allograft loss of serum calcium concentration, serum phosphate concentration, and calcium phosphate concentration product.

	HR (95% CI)	
	Univariate	Multivariate
Serum calcium concentration (in mmol/l)		
Quintile 1 (≤2.25)	1.0 (referent)	1.0 (referent)
Quintile 2 (2.26–2.32)	0.61 (0.39–0.95)	0.90 (0.57–1.44)
Quintile 3 (2.33–2.40)	0.35 (0.21–0.57)	0.52 (0.31–0.87)
Quintile 4 (2.41–2.49)	0.45 (0.28–0.72)	0.68 (0.41–1.12)
Quintile 5 (≥2.50)	0.40 (0.25–0.65)	0.59 (0.36–0.97)
Serum phosphate concentration (in mmol/l)		
Quintile 1 (≤0.84)	1.0 (referent)	1.0 (referent)
Quintile 2 (0.85–0.96)	1.31 (0.65–2.63)	1.34 (0.66–2.72)
Quintile 3 (0.97–1.08)	1.88 (0.98–3.61)	1.60 (0.83–3.10)
Quintile 4 (1.09–1.22)	2.25 (1.19–4.25)	1.67 (0.87–3.23)
Quintile 5 (≥1.23)	7.08 (3.98–12.60)	3.30 (1.75–6.23)
Serum calcium phosphate product (in $mmol^2/l^2$)		
Quintile 1 (≤2.00)	1.0 (referent)	1.0 (referent)
Quintile 2 (2.00–2.29)	1.58 (0.82–3.04)	1.37 (0.71–2.64)
Quintile 3 (2.29–2.57)	1.30 (0.66–2.55)	1.11 (0.56–2.22)
Quintile 4 (2.57–2.89)	2.45 (1.34–4.50)	1.71 (0.92–3.19)
Quintile 5 (≥2.90)	5.98 (3.41–10.49)	2.51 (1.35–4.66)

Multivariate models additionally adjusted for recipient age, gender, estimated glomerular filtration rate, diabetic nephropathy, donor gender, and time from first renal replacement therapy to transplantation; multivariate models contain only one calcium/phosphate parameter (in categories) at a time.

compared with those in the lowest quintile. These associations remained significant after multivariate adjustment (HR = 0.59; 95% CI: 0.36–0.97).

Compared with analyses on the composite end point (Table 5), we found even more pronounced associations between serum phosphate concentration and the risk for death-censored allograft loss, with a doubling of risk for KTRs whose phosphate concentration was between 1.09 and 1.22 mmol/l (HR = 2.25; 95% CI: 1.19–4.25), and a sevenfold increased risk for KTR whose serum phosphate concentration was ≥1.23 mmol/l (HR = 7.08; 95% CI: 3.98–12.60). Once again, the magnitude of the association was reduced with multivariate analysis, but the highest quintile of serum phosphate concentration was still associated with triple the risk for death-censored kidney allograft loss compared with the lowest quintile (HR = 3.30; 95% CI: 1.75–6.23). The results for the $CaPO_4$ concentration product were similar with strong associations in univariate analysis for product concentrations ≥2.57 $mmol^2/l^2$, and a 2.5-fold risk for KTR with a concentration product ≥2.90 $mmol^2/l^2$ after multivariate adjustment (HR = 2.51; 95% CI: 1.35–4.66).

Discussion

In this prospective study of stable KTR, we found associations between concentrations of serum calcium, serum phosphate and $CaPO_4$ product and the risk of kidney allograft loss. These findings arose from carefully adjusted multivariate models and remained independent of renal function and several other potentially confounding factors. Whereas higher levels of serum calcium seemed to be associated with a reduced risk of allograft loss, higher concentrations of phosphate and $CaPO_4$ product were associated with an increased risk. The magnitude of these associations was even more pronounced when patients were censored at death (death-censored allograft loss). By contrast, no independent associations were found between these electrolytes and all-cause mortality. To our knowledge, this is the first study of the long-term relationship between parameters of mineral metabolism and relevant outcomes in KTR.

Hyperphosphatemia is highly prevalent among patients with end-stage renal disease and has been linked to vascular calcification and increased mortality risk in hemodialysis patients. Block *et al.* [2] studied 6407 prevalent hemodialysis patients and found a relative risk of death of 1.27 above a serum phosphate concentration of 6.5 mg/dl (=2.09 mmol/l) that did not change after multivariate adjustment including nutritional parameters or patient non-compliance. Cause-specific mortality could not be established in their study, but a subsequent study of 12 833 prevalent hemodialysis patients explored the specific hypothesis that the increased mortality risk associated with elevated phosphate concentrations was primarily attributable to cardiac rather than to non-cardiac

events [11]. Indeed, patients with serum phosphate concentrations >6.5 mg/dl (=2.09 mmol/l) had a 41% greater mortality risk from coronary artery disease compared with patients below that value, suggesting that phosphate concentrations >6.5 mg/dl (=2.09 mmol/l) may be cardiotoxic for patients on chronic hemodialysis. Of note, the phosphate concentrations that were found to be associated with increased risk in this study were substantially larger than the concentrations found in our sample of KTR.

A recent analysis by Kestenbaum *et al.* [12] studied the association between serum phosphate concentration and mortality risk in 3490 patients with CKD; the study population exhibited a high prevalence of diabetes and coronary artery disease. In this retrospective study of US veterans, who had at least two abnormal outpatient serum creatinine and serum phosphate measurements, phosphate levels >3.5 mg/dl (=1.13 mmol/l) were associated with a significantly increased risk for death after multivariate model adjustment. The observed mortality risk increased linearly with each subsequent 0.5 mg/dl increase in phosphate levels. Another smaller investigation by Menon *et al.* [13] studied 840 participants of the Modification of Diet in Renal Disease study, who had CKD at stages 3 and 4 and whose serum calcium and phosphate levels were measured at baseline. In this study, neither serum phosphate concentration nor $CaPO_4$ concentration product were associated with all-cause or cardiovascular mortality, but the authors recognized the limited statistical power of their analysis. Our study mirrors the design and the results of this study, with the main difference that Menon studied patients with CKD and our study investigated KTR patients. Thus far, no specific analyses on the prognostic role of calcium or phosphate concentrations in KTR have been published.

Successful kidney transplantation has been thought to correct the endocrine and metabolic imbalances and the main abnormalities responsible for secondary hyperparathyroidism associated with dialysis treatment within a few months after transplantation [14], but persistent disease has emerged in a significant number of KTR as late as 1 year after transplantation despite adequate renal function [15], and a subgroup of these patients requires subsequent parathyroidectomy. Acute renal functional impairment from mechanisms yet to be determined has been reported in KTR following parathyroidectomy [16,17].

There are numerous reports about bone abnormalities resulting in bone loss and fractures related to underlying disturbed mineral metabolism [5,18,19]. However, data about the relationship of serum calcium, phosphate and/or $CaPO_4$ concentration product or parathyroid hormone as risk factors for mortality in KTR are absent and those on graft survival are very limited. Only one relatively larger study investigated calcium levels as a potential risk factor for delayed graft function in 585 KTR patients [20]. This study showed that higher serum calcium levels were independently associated with delayed graft function but not with microscopic nephrocalcinosis. Phosphate levels did not correlate with the occurrence of delayed graft function and neither did the $CaPO_4$ concentration product. The sole endpoint of this study was delayed graft function within the first week after transplantation. A very recent study by Gwinner *et al.* [21] examined calcifications in 586 serial protocol biopsies of 213 patients testing whether calcification is related to parameters of calcium homoeostasis and analyzing a relation between calcification and graft function at 1 year. Patients with calcifications had significantly higher serum calcium and parathyroid hormone levels. Serum phosphate levels were comparable between patients with versus without calcifications. The incidences of rejection, chronic changes, or acute tubular lesions did not differ between both groups, but the results indicated that in patients with calcification, impairment of graft function at 1 year after transplantation was correlated with high parathyroid hormone levels. Interestingly, correlation of calcium and parathyroid hormone levels suggested that hyperparathyroidism in patients with calcification was not secondary in response to low calcium levels, again suggesting a dysregulation of parathyroid hormone [14]. In this context the results of our cohort, in which high serum calcium concentrations were not associated with increased mortality, but where the observed association with allograft survival was protective, if anything, after 6 years of observation seem to be compatible with the findings in the study by Gwinner *et al.*

The presence of associations between the study exposure variables and the outcome of allograft loss deserves further consideration. While we controlled for estimated renal function in our multivariate models, markers of calcium phosphate homeostasis are correlated with renal allograft function (see Table 3). Strictly interpreting the results, for any given level of eGFR, calcium concentration, phosphate concentration, and their product are each independently associated with allograft loss. The causality of these associations, however, is questionable. It is likely, for example, that for any given level of eGFR, patients with a higher phosphate concentration will be more likely to be advised to return to dialysis, one component of the outcomes definition, than patients with identical eGFR who have lower phosphate concentrations. This hypothesis is clearly supported by the fact that the estimated associations are more pronounced in death-censored analyses compared with the analyses of the combined endpoint.

These considerations lead us to acknowledging the limitations of this study. Information on other important parameters in calcium phosphate homeostasis such as parathyroid hormone levels or vitamin D metabolites was unavailable for study. Evaluating these factors appears necessary to fully understand the pathophysiological mechanisms of disease. Further, no information was available on vitamin D treatment or intake of calcium or phosphate supplements, either at baseline or during follow-up. Repeat measures of renal function, information on acute rejection episodes, or protocol biopsies by which information of calcification would have been obtained were also unavailable for study. This precluded us from modeling the association between an abnormal mineral metabolism and the outcome of difference in renal function or other important outcomes in this sample. Although prospective, our analyses are still of observational nature; thus, residual confounding by unmeasured factors is possible. As we studied prevalent rather than incident KTR, we cannot rule out time-related biases such as survival bias. We did not have any information on smoking status or blood pressure. Finally specific data on cardiovascular cause of death or occurrence of non-fatal myocardial infarction was not available for study. Inclusion of non-fatal study endpoints would certainly have increased the statistical power to detect any truly existing associations.

Further investigation and confirmation of these surprising study results are needed. Specifically, future studies with more accurate and repeated measurements of allograft function are warranted. Greater appreciation of the prevalence and a better understanding of the pathophysiological mechanisms and outcomes of an abnormal post-transplant mineral metabolism may pave the way to targeted intervention, improved care, and better long-term outcomes of patients after kidney transplantation.

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