Received: 15 May 2020 | Revised: 8 November 2020 | Accepted: 9 December 2020

DOI: 10.1111/ajt.16457

ORIGINAL ARTICLE

AJT

Association of time-updated plasma calcium and phosphate with graft and patient outcomes after kidney transplantation

Willemijn Y. van der Plas[1] | **António W. Gomes Neto**[2] | **Stefan P. Berger**[2] | **Robert A. Pol**[1] | **Schelto Kruijff**[1] | **Stephan J. L. Bakker**[2,3] | **Martin H. de Borst**[2]

[1]Department of Surgery, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

[2]Department of Internal Medicine Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

[3]TransplantLines Biobank and Cohort Study, Groningen, The Netherlands

Correspondence
Martin H. de Borst, Department of Internal Medicine Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.
Email: m.h.de.borst@umcg.nl

Disturbances in calcium-phosphate homeostasis are common after kidney transplantation. We aimed to assess the relationship between deregulations in plasma calcium and phosphate over time and mortality and death-censored graft failure (DCGF). In this prospective cohort study, we included kidney transplant recipients with ≥2 plasma calcium and phosphate measurements. Data were analyzed using time-updated Cox regression analyses adjusted for potential confounders including time-updated kidney function. We included 2769 patients (mean age 47 ± 14 years, 42.3% female) with 138 496 plasma calcium and phosphate levels (median [IQR] 43 [31–61] measurements per patient). During follow-up of 16.3 [8.7–25.2] years, 17.2% developed DCGF and 7.9% died. Posttransplant hypercalcemia was associated with an increased risk of mortality (1.63 [1.31–2.00], $p < 0.0001$), but not with DCGF. Hyperphosphatemia was associated with both DCGF (2.59 [2.05–3.27], $p < .0001$) and mortality (3.14 [2.58–3.82], $p < .0001$). Only the association between hypercalcemia and mortality remained significant in sensitivity analyses censored by a simultaneous eGFR <45 mL/min/1.73 m^2. Hypocalcemia and hypophosphatemia were not consistently associated with either outcome. Posttransplant hypercalcemia, even in the presence of preserved kidney function, was associated with an increased mortality risk. Associations of hyperphosphatemia with DCGF and mortality may be driven by eGFR.

KEYWORDS
clinical research/practice, health services and outcomes research, hyperparathyrodism, kidney (allograft) function/dysfunction, kidney transplantation/nephrology

1 | BACKGROUND

Disturbances in calcium-phosphate metabolism are common in patients with advanced chronic kidney disease (CKD), and have been associated with an increased risk of cardiovascular disease and mortality.[1-3] After kidney transplantation, mineral metabolism is at least partly restored with improved kidney function, although abnormalities may persist on the long term in considerable numbers of patients.

Abbreviations: CKD, chronic kidney disease; CMV, cytomegalovirus; DCGF, death-censored graft failure; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; ERA-EDTA, European Renal Association – European Dialysis and Transplant Association; ESRD, end-stage renal disease; FGF, fibroblast growth factor; HLA, human leukocyte antigen; IQR, interquartile range; METc, medical ethics committee; MICE, multiple imputation by chained equations; N, number; PRD, primary renal disease; PTH, parathyroid hormone; SD, standard deviation; UMCG, University Medical Center Groningen.

Irrespective of graft function, high bone turnover is common within the first months after transplantation.[4] On the first day after transplantation, plasma calcium decreases in 41% of patients.[5] However, hypercalcemia is also common (up to 52%) in the first postoperative months,[4] which may at least partly be related to persistently increased parathyroid hormone (PTH) levels in patients with preexisting end-stage renal disease (ESRD)-related hyperparathyroidism.[5] Likewise, plasma phosphate concentrations are often disturbed after kidney transplantation. We previously found that 47% of kidney transplant recipients develop severe hypophosphatemia (<0.5 mmol/L or <1.55 mg/dL), which mostly occurred during the first 3 months after transplantation.[6] Posttransplant hypophosphatemia is likely caused by inappropriately high PTH and fibroblast growth factor (FGF)-23 combined with recovered kidney function in the early posttransplant stage, driving phosphaturia.[7] In contrast, hyperphosphatemia may also occur, particularly with impaired graft function.

The prognostic implications of posttransplant deregulated calcium and phosphate homeostasis for patient and graft outcomes are unclear. Previous studies have linked a higher plasma calcium level with an increased risk of delayed graft function (DGF) and chronic allograft dysfunction.[8,9] Higher plasma phosphate levels have been associated with an increased risk of all-cause mortality and death-censored graft loss, whereas hypophosphatemia might have a favorable impact on graft and patient outcomes.[6,10,11] However, these analyses used only a single calcium or phosphate measurement or a mean of multiple measurements, while time-dependent variation has not been taken into account.

We used a large real-world dataset from the Transplantlines cohort study (NCT03272841) to analyze time-updated plasma calcium and phosphate levels, accounting for potential confounders including time-updated estimated glomerular filtration rate (eGFR), to investigate whether deregulated plasma calcium and phosphate are associated with adverse graft and patient outcomes.[12]

2 | MATERIALS AND METHODS

2.1 | Patient population

All patients who underwent a kidney transplantation at the University Medical Center Groningen (UMCG), The Netherlands, between March 1970 and January 2016 were considered for inclusion in this study. Of patients who had undergone multiple kidney transplantations, only data regarding the first kidney transplantation were included. Only patients with at least two simultaneous plasma calcium and phosphate measurements at any point in time were included for this study. Patients with ≤1 calcium and phosphate measurements post-KTx, with graft failure or mortality <3 months after transplantation or with missing follow-up data were excluded. Measurements obtained during severely impaired kidney function (eGFR <15 mL/min/1.73 m^2) or during intensive care unit admission were not taken into account. This study was approved by the local medical ethics committee (METc 2014/077). The study was performed in accordance with the Declaration of Helsinki and the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

2.2 | Laboratory measurements

Routine laboratory measurements were extracted from the laboratory information system of the University Medical Center Groningen. Plasma calcium, phosphate, creatinine, and albumin concentrations were measured at all outpatient department visits. Plasma calcium corrected for albumin concentration was calculated according to the following formula: corrected calcium (mmol/L) = measured calcium (mmol/L) + (0.025 * (40 – [albumin (g/L)]). Reference values for plasma-corrected calcium were 2.20–2.60 mmol/L (8.8–10.4 mg/dL) and for plasma phosphate 0.70–1.50 mmol/L (2.17–4.64 mg/dL). At each individual measurement, patients were classified as having hypo-, normo-, or hypercalcemia and hypo-, normo-, or hyperphosphatemia according to these definitions. All routine measurements before March 2006 were performed on the Merck Mega Analyzer (Merck); measurements after March 2006 were performed on the Roche Modular (Roche Ltd.). Laboratory measurements prior to March 2006 were converted according to equations listed in Table S1. The PTH assay used in our hospital changed in 2006 from Nichols Institute Diagnostics (San Juan Capistrano) to Immulite 2500 (Siemens Healthcare Diagnostics) and Cobas e601 immunology analyzer (Roche Diagnostics). Therefore, PTH measurements after 2006 were converted using an in-house established conversion formula.[6] The PTH measurement closest to the KTx date was used for analyses. Reference range for PTH is <7.8 pmol/L (74 pg/mL). Creatinine-based eGFR was calculated according to the CKD Epidemiology Collaboration Equation (EPI) equation.[13] All other measurements were performed using standard laboratory techniques.

2.3 | Follow-up

All patients who receive a kidney transplantation in our center undergo a standardized follow-up regime. Patients receive a standardized immunosuppression protocol, which comprises triple therapy with tacrolimus or cyclosporine, in combination with mycophenolate mofetil and corticosteroids, as previously reported.[6,14] Shortly after kidney transplantation, patients visit the outpatient department weekly. The frequency of visits is tapered to every 4–6 weeks during the first year after transplantation, and at least four times a year after the first year. End of follow-up was March 2016. As part of the Transplantlines registry, donor and recipient characteristics were collected.[12] Primary cause of ESRD was categorized according to the ERA-EDTA Registry Coding System.[15]

2.4 | Study endpoints

The primary outcomes of this study were death-censored graft failure (DCGF), defined as return to dialysis or retransplantation, and all-cause mortality. Mortality data were verified with the Dutch Municipal Registry Office. We analyzed the impact of both posttransplant calcium levels and phosphate levels on DCGF and mortality.

2.5 | Statistical analyses

Continuous variables are reported as mean ± standard deviation (SD) for normally distributed variables or median with interquartile range (IQR) for nonnormally distributed variables. Variable distribution was assessed by plotting histograms. Categorical variables are expressed as number (*n*) and percentage (%). Skewed variables were log-transformed where appropriate. Of all available laboratory measurements, the mean plasma calcium or phosphate per patient per month was calculated. Distribution of these mean monthly values above or below the reference range was investigated.

We performed time-updated statistical analyses, where age, body weight, eGFR, proteinuria, number of antihypertensive drugs, systolic blood pressure, immunosuppressive drug use, and laboratory values were updated at the time of each single calcium or phosphate measurement, when available. In case of missing data, the last available observation was carried forward. For example, when a subsequent calcium/phosphate measurement was done, but no new information on systolic blood pressure was available, the previous systolic blood pressure value was used. The pretransplant PTH value closest to the transplantation date was included in subanalyses. We handled remaining missing data using multiple imputation of variables with less than 10% missing data. Data of the following variables were imputed using multiple imputation by chained equations (MICE) in R with five imputations: primary renal disease (PRD), donor age, donor sex, donor status, dialysis modality, recipient cytomegalovirus (CMV) infection, cold ischemia time, warm ischemia time, number of human leucocyte antigen (HLA) mismatches, eGFR, proteinuria, number of antihypertensive drugs and systolic blood pressure, using age, sex, plasma-corrected calcium concentration, plasma phosphate level, immunosuppressive drug use, dialysis vintage, era of transplantation, and delayed graft function (DGF) as auxiliary variables. DGF was defined as the need for dialysis within the first 7 days posttransplant. Pooled results of the statistical analyses are reported according to Rubin's rules as main analyses in this manuscript.[16]

First, we performed univariable Cox regression analyses to assess the impact of both calcium and phosphate values as categorical variables (hypo-, normo-, or hypercalcemia and hypo-, normo-, or hyperphosphatemia, respectively) on DCGF and mortality. Next, we performed time-updated multivariate Cox regression analyses adjusting for potential confounders. We cumulatively adjusted for age and sex (Model 2), time-updated eGFR and proteinuria after transplantation (Model 3), donor age, sex and status, cold and warm ischemia time, number of HLA mismatches, PRD, CMV infection of the recipient, number antihypertensive drugs, systolic blood pressure, plasma phosphate/corrected calcium, dialysis vintage, decade of transplantation, immunosuppressive drug use, and DGF (Model 4).

The associations of calcium and phosphate with DCGF and mortality were further investigated using time-updated restricted cubic splines with three knots, at the 10^{th}, 50^{th}, and 90^{th} percentile. The median of the variable of interest was indicated as reference for all spline plots. Cubic spline graphs are presented after full adjustment similar to the Cox regression analyses.

We performed a competing risk analysis by taking graft failure into account when assessing the risk of mortality (graft failure-censored mortality).[17] Furthermore, we performed several sensitivity analyses. First, we repeated the analyses with the non-imputed dataset. Second, analyses were rerun with the imputed dataset after excluding the 0.5% highest and lowest calcium and phosphate values. Third, we assessed whether pretransplant PTH affected the associations of plasma calcium and phosphate levels with the risk of DCGF and mortality in a subgroup with pretransplant PTH data available. Fourth, we assessed the relationship between calcium or phosphate with mortality, censored for graft failure. Fifth, we repeated all analyses in a subgroup of patients with an eGFR ≥45 mL/min/1.73 m2 at 1-year posttransplant. Finally, we censored plasma calcium and phosphate data at any time point when the simultaneously measured eGFR was <45 mL/min/1.73 m^2; the calcium and phosphate value of the previous measurement with a simultaneous eGFR ≥45 mL/min/1.73 m^2 was carried forward. Patients who never reached an eGFR ≥45 mL/min/1.73 m^2 were excluded from this analysis.

Statistical analysis was performed using SPSS Statistics version 25.0 (IBM Corporation) and R version 3.2.6 (Vienna, Austria); a P-value of <.05 was considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics

We included 2769 patients who underwent their first kidney transplantation between 1970 and 2016 (Figure 1). Baseline characteristics are shown in Table 1. Mean recipient age was 47 ± 14 years and 42.3% were female. Mean BMI prior to transplantation was 25 ± 4 kg/m^2. Donor age was 43 ± 16 years and the majority of donors were postmortal donors (71.5%); 537 (19.4%) patients underwent a preemptive transplantation. Pretransplant PTH levels were available in 1,412 patients (51%). Median pretransplant plasma PTH concentration was 26.1 (11.0–47.7) pmol/L (246 [104–450] pg/mL), with the majority of patients (52.3%) having PTH levels between two and nine times the upper limit of normal. Median follow-up duration was 16.3 (8.7–25.2) years.

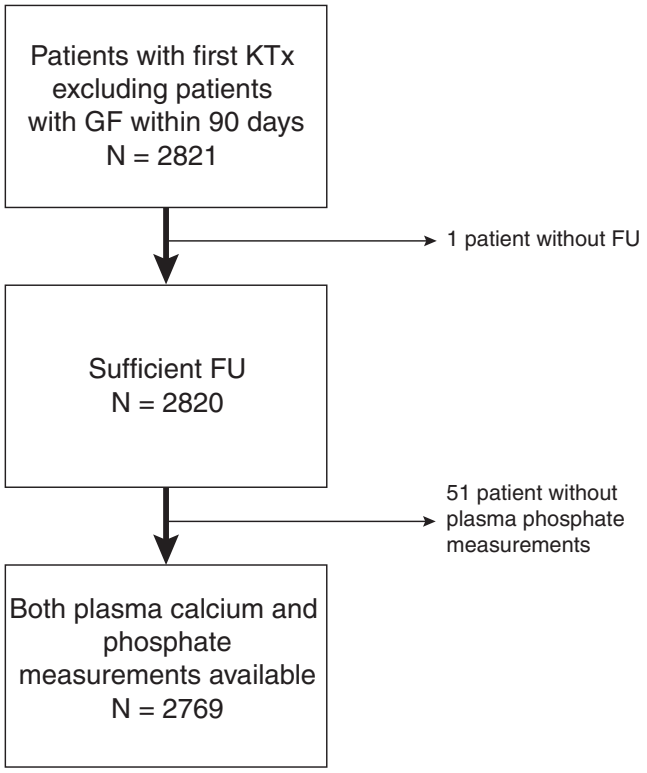

FIGURE 1 Flowchart of patient cohort. KTx, kidney transplantation; GF, graft failure; FU, follow-up

3.2 | Plasma calcium and phosphate over time

Overall, 138 496 plasma calcium and phosphate measurements were available from 2769 patients, with a median of 43 (31–61) measurements per patient. The intraindividual mean plasma-corrected calcium concentration was 2.39 ± 0.18 mmol/L (9.56 ± 0.05 mg/dL). Within the first year posttransplant, up to one third of patients had at least one monthly mean calcium value outside the reference range; hypercalcemia was slightly more common than hypocalcemia (Figure 2A). This difference disappeared >1 year. The intraindividual mean plasma phosphate concentration was 1.01 ± 0.39 mmol/L (3.13 ± 1.21 mg/dL). Within the first month after transplantation, 24% of all phosphate measurements was below the lower limit of normal, whereas 16% of the measurements was above the reference range in the first month (Figure 2B).

3.3 | Posttransplant plasma calcium, phosphate, and the risk of DCGF

During the median follow-up of 6.4 (3–13) years, 477 patients (17.2%) developed DCGF. Upon univariable analysis, both hypo- and hypercalcemia were associated with an increased risk of DCGF, compared with normocalcemia; however, both associations lost significance upon multivariable adjustment (Table 2 and Figure 3). The results remained similar in sensitivity analyses investigating the original data prior to imputation (Table S2), adjusting for PTH in a subgroup with PTH levels available (Table S4), after excluding 0.5% highest and lowest plasma calcium levels (Table S6), and in a subgroup with patients with eGFR ≥45 mL/min/1.73 m^2 at 1-year posttransplant (Table S8).

Patients with hypophosphatemia had a lower risk of developing DCGF compared with normophosphatemia in univariable analysis (Table 3, Figure 4). However, in the fully adjusted model, including adjustment for time-updated eGFR, hypophosphatemia was associated with a higher DCGF risk (fully adjusted HR 2.17 [95% CI 1.24–3.78], p = .007). The association between hypophosphatemia and DCGF persisted in a sensitivity analysis after excluding the 0.5% highest and lowest plasma phosphate values (Table S7), however could not be reproduced when analyzing the original (preimputation) data, or in subgroups of patients with available pretransplant PTH levels or patients with eGFR ≥45 mL/min/1.73 m^2 at 1-year posttransplant (Tables S3, S5, and S9, respectively).

Hyperphosphatemia was strongly associated with an increased risk of DCGF, compared with normophosphatemia (fully adjusted HR 2.49 [95% CI 2.05–3.27], p < .0001), as depicted in Table 3 and Figure 4. The association between hyperphosphatemia and DCGF risk was also observed in sensitivity analyses of the original data (Table S3), in patients with PTH measurements available (Table S5), after exclusion of the 0.5% highest and lowest plasma phosphate levels (Table S7), and in patients with an eGFR ≥45 mL/min/1.73 m^2 at 1-year posttransplant (Table S9). However, in the most stringent sensitivity analysis, excluding all measurements with a concurrent eGFR <45 mL/min/1.73 m^2, hyperphosphatemia was no longer associated with DCGF (Table S11).

3.4 | Posttransplant plasma calcium, phosphate, and the risk of mortality

During follow-up of 9 (5–15) years, 1050 patients (37.9%) died. In univariable analysis, hypocalcemia was associated with a higher mortality risk compared with normocalcemia (HR 1.64 [95% CI 1.39–1.93], p < .0001), however significance was lost upon multivariate adjustment (Table 2). Hypercalcemia was also associated with a higher risk of mortality compared to normocalcemia, which persisted after multivariable adjustment (HR 1.63 [95% CI 1.31–2.00], p < .0001, Table 2 and Figure 3). The association between hypercalcemia and mortality persisted in all sensitivity analyses (Tables S2, S4, S6, S8). Similar findings were obtained in a very strict sensitivity analysis where we censored all calcium and phosphate measurements with a simultaneous eGFR <45 mL/min/1.73 m^2 (Table S10).

Hypophosphatemia tended to be associated with a lower risk of death in univariable analyses; however, this association did not remain statistically significant in the fully adjusted model (HR 0.88 [95% CI 0.69–1.15], p = .36), as shown in Table 3 and Figure 4. Hyperphosphatemia was associated with a threefold risk of death, compared to normophosphatemia (fully adjusted HR 3.14 [95% CI 2.58–3.82], p < .001). Similar findings were observed in sensitivity analyses (Tables S3, S5, S7, S9). Upon censoring all calcium

TABLE 1 Baseline characteristics of the cohort

Baseline characteristics of the cohort	Total population *N* = 2769
Patient characteristics	
Female sex, n (%)	1171 (42.3)
Age, years	47 ± 14
Height, cm	173 ± 9
Missing	387 (14.0)
BMI (kg/m^2)	24.5 ± 4.4
Missing	451 (16.3)
Primary ESRD cause, n (%)	
Glomerulonephritis	599 (21.6)
Interstitial-nephritis	335 (12.1)
Cystic kidney disease	485 (12.1)
Other congenital and hereditary kidney disease	126 (4.6)
Renal vascular disease, excluding vasculitis	268 (9.7)
Diabetes mellitus	195 (7.0)
Other multisystem diseases	111 (4.0)
Other	76 (2.7)
Unknown	563 (20.3)
Missing	11 (0.4)
Preemptive transplantation, n (%)	537 (19.4)
Dialysis duration, mo	33.0 (18.0 – 54.0)
Missing	33 (1.0)
Dialysis type, n (%)	
Hemodialysis	1439 (52.0)
Peritoneal dialysis	783 (28.3)
Missing	10 (0.4)
Cytomegalovirus infection, n (%)	
Primary	233 (8.4)
Secondary	422 (15.2)
No	1836 (66.3)
Other CMV infections	13 (0.5)
Missing	265 (9.6)
Donor characteristics	
Female sex donor, n (%)	1291 (46.6)
Missing	8 (0.3)
Age donor, yrs	43 ± 16
Missing	10 (0.4)
Donor status, n (%)	
Deceased	1981 (71.5)
Living	787 (28.4)
Missing	1 (0.0)
Transplantation characteristics	
Cold ischemia time, h	15.4 (3.0 – 22.2)
Missing	73 (2.6)
Second warm ischemia time, min	39 ± 11
Missing	46 (1.6)
Number of HLA mismatches	
0	406 (14.7)
1	295 (14.3)
2	688 (24.8)
3	658 (23.8)
>3	519 (18.7)
Missing	103 (3.7)

Data are presented as mean (SD) or median [first to third quartiles] unless otherwise noted.

Abbreviations: BMI, body mass index; CMV, cytomegalovirus; ESRD, end-stage renal disease; HLA, human leukocyte antigen.

and phosphate measurements with a simultaneous eGFR <45 mL/min/1.73 m^2, the association between hyperphosphatemia and mortality was borderline significant (HR 1.67 [95% CI 0.97–2.88], p = .07; Table S11).

The majority of patients (76.4%) died with a functioning kidney graft. An additional analysis of the risk of graft failure-censored mortality led to similar results for calcium (Table S12) and phosphate (Table S13).

4 | DISCUSSION

Calcium-phosphate homeostasis is frequently disrupted after kidney transplantation. During the first year of follow-up, up to 33% of patients in our study had at least one monthly mean calcium value outside the reference range and 45% had minimally one monthly mean phosphate measurement outside the reference range. In this study, we found that patients with posttransplant hyperphosphatemia have a significantly increased risk of DCGF compared with normophosphatemia, after adjustment for potential confounders including time-updated eGFR. In addition, patients with hypercalcemia or hyperphosphatemia had an increased mortality risk. Interestingly, the association between hypercalcemia and mortality remained significant in sensitivity analyses censored by a simultaneous eGFR <45 mL/min/1.73 m^2, while the association between hyperphosphatemia and mortality showed a borderline significant trend. This implicates that even in individuals with preserved kidney function, hypercalcemia is associated with mortality, while confounding by eGFR strictly cannot be excluded for the other associations. Hypocalcemia and hypophosphatemia were not consistently associated with either outcome. Our findings at least in part provide support to current KDIGO guidelines, stating that "it is reasonable to manage abnormalities in calcium and phosphate as for patients with CKD stages 3–5," whereas this statement was not graded due to limited supporting evidence.[18]

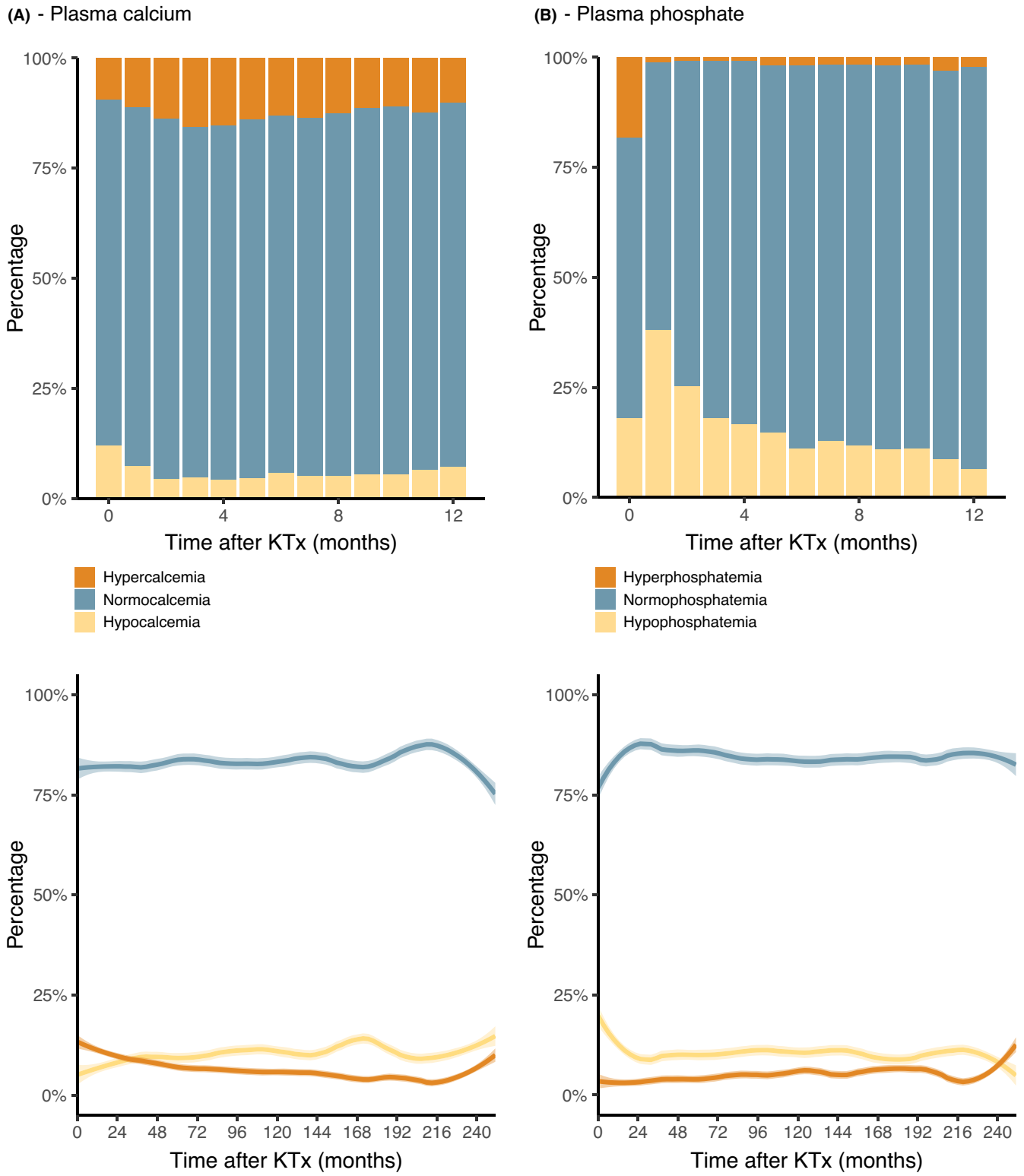

FIGURE 2 Distribution of proportion of patients with a monthly mean plasma calcium (A) or plasma phosphate (B) within, below, or above the reference range for the first 12 months (upper panel) and during long-term follow-up (lower panel) [Color figure can be viewed at wileyonlinelibrary.com]

Several factors may contribute to the disturbances in mineral metabolism observed after kidney transplantation. In the early posttransplant stage, restored kidney function may partly resolve the disturbances that arose during the development of kidney failure. Hyperparathyroidism related to ESRD plays an important role contributing to hypercalcemia before and after kidney transplantation.[5,18-20] After transplantation, ESRD-related hyperparathyroidism resolves in up to 57% of patients within

TABLE 2 Cox regression analysis of plasma calcium versus death-censored graft failure (DCGF) and mortality

	DCGF			Mortality		
	Pooled HR	95% CI	*p* value	Pooled HR	95% CI	*p* value
Model 1						
Hypocalcemia	4.58	3.77 – 5.56	<.0001	1.64	1.39 – 1.93	<.0001
Normocalcemia	1.00 (ref)	-	-	1.00 (ref)	-	-
Hypercalcemia	0.49	0.29 – 0.84	.009	1.72	0.40 – 2.11	<.0001
Model 2						
Hypocalcemia	4.53	3.73 – 5.50	<.0001	1.78	1.51 – 2.09	<.0001
Normocalcemia	1.00 (ref)	-	-	1.00 (ref)	-	-
Hypercalcemia	0.54	00.32 – 0.93	.03	1.49	1.21 – 1.84	.0002
Model 3						
Hypocalcemia	1.17	0.95 – 1.14	.33	1.32	1.12 – 1.57	.001
Normocalcemia	1.00 (ref)	-	-	1.00 (ref)	-	-
Hypercalcemia	0.67	0.38 – 1.17	.16	1.51	1.22 – 1.59	.0001
Model 4						
Hypocalcemia	1.02	0.82 – 1.27	.79	1.09	0.91 – 1.36	.32
Normocalcemia	1.00 (ref)	-	-	1.00 (ref)	-	-
Hypercalcemia	0.77	0.44 – 1.35	.71	1.63	1.31 – 2.00	<.0001
	Number of events: 477			Number of events: 1050		

Model 1: unadjusted analysis.

Model 2: adjusted for age and sex.

Model 3: Model 2 plus time-updated eGFR and proteinuria.

Model 4: Model 3 plus donor age, donor sex and donor status, cold and warm ischemia time, number of HLA mismatches, primary renal disease, CMV infection of the recipient, antihypertensive drug use, systolic blood pressure, plasma phosphate, dialysis vintage, decade of transplantation, immunosuppressive drug use, delayed graft function.

Abbreviations: CI, confidence interval; DCGF, death-censored graft failure; HR, hazard ratio.

2 years after transplantation.[21] Still, both hypo- and hypercalcemia are relatively common: in our cohort, 11.1% and 10.9% of our patients were hypo- or hypercalcemic, respectively, in the first 30 days posttransplant. Additionally, it has been postulated that adynamic bone disease in combination with tubular reabsorption of calcium could be another cause of hypercalcemia after transplantation, and so may the use of calcium or vitamin D supplements.[22] On the other hand, an abrupt cessation of calcium-containing phosphate binders and vitamin D analogs might partly explain a sudden decrease in plasma calcium levels postoperatively in a small proportion of posttransplant patients.[5] On top of this, already low pretransplant PTH levels due to parathyroidectomy might drop even further after transplantation, causing hypocalcemia.[23] Increased plasma PTH levels prior to transplant have been shown to be protective for the development of posttransplant hypocalcemia.[5] Hyperphosphatemia is common in ESRD patients due to impaired kidney function.[24] In contrast, hypophosphatemia may develop due to relatively high FGF-23 and PTH levels after transplantation, in the context of restored kidney function allowing massive phosphaturia.[7,25] Other contributing factors might include relative vitamin D deficiency, glucocorticoid use, and other immunosuppressive drugs such as cyclosporine.[26]

Previous studies suggested that hypercalcemia might lead to nephrocalcinosis, which impairs renal function in several ways, including tubular obstruction and back-leak, vasoconstriction, and hypoxia.[9] Interestingly, we did not find a significant association of hypercalcemia with DCGF, in line with Moore and colleagues.[10] At the same time, chronic hypercalcemia may promote vascular calcification, which may enhance cardiovascular risk.[27-29] Interestingly, successful kidney transplantation seems to slow the progression of coronary calcification in some, but not all patients.[29] Hypercalcemia has been associated with an increased risk of mortality in CKD and dialysis patients, but data in kidney transplant recipients have been scarce.[10] Our study showed a consistent association between hypercalcemia and an increased all-cause mortality risk. Hypocalcemia, on the other hand, might induce electrocardiographic changes, such as prolongation of the QTc interval, and has been associated with an increased risk of mortality in ESRD patients.[30-33] We did not observe a significant relationship between hypocalcemia and clinical outcomes in this study.

Several previous studies have demonstrated the associations of plasma phosphate with an increased risk of (death-censored) graft failure and all-cause mortality.[10,11,34-36] Mechanistically, hyperphosphatemia, particularly when combined with higher calcium levels, may promote calcium-phosphate crystal deposition in the tubular

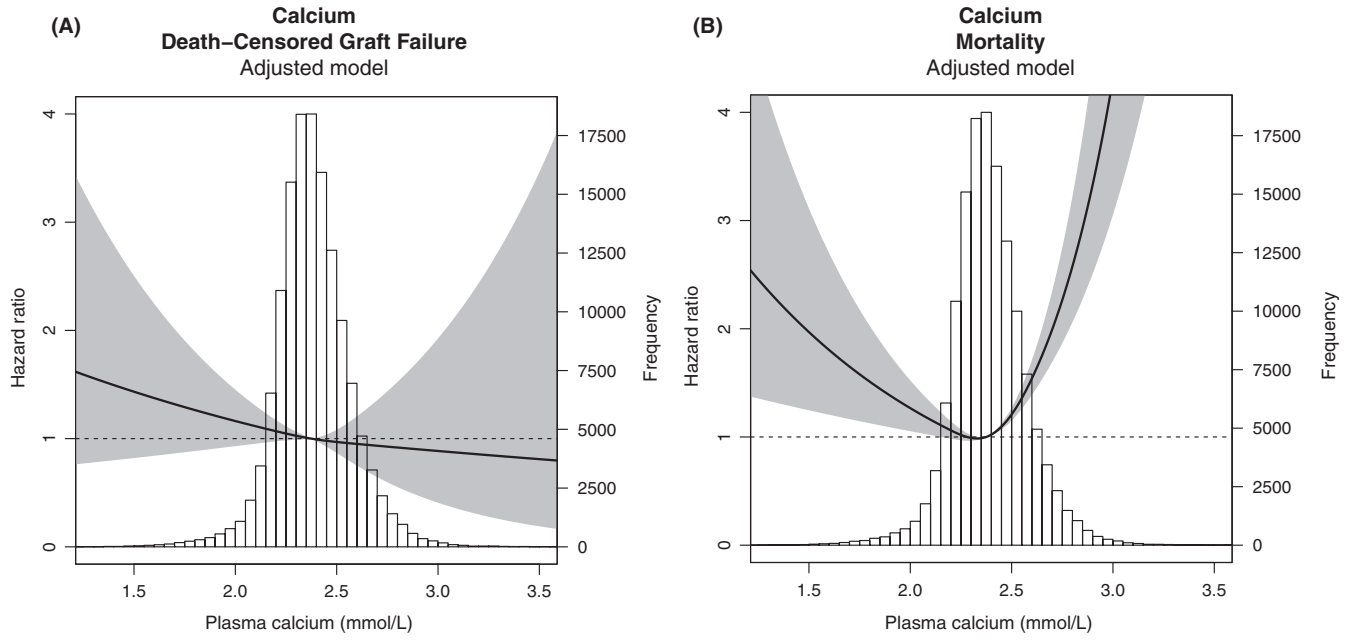

FIGURE 3 Spline curves illustrating the association between plasma calcium levels and the risk of (A) death-censored graft failure and (B) all-cause mortality. Models are on the basis of a cubic spline term (restricted cubic spline) with three knots. The solid lines represent the fully adjusted hazard ratios (HRs) for death-censored graft failure (Cox regression model 4) and all-cause mortality (Cox regression model 4). The gray areas represent the 95% confidence intervals of the HRs

TABLE 3 Cox regression analysis of plasma phosphate versus death-censored graft failure (DCGF) and mortality

	DCGF			Mortality		
	Pooled HR	**95% CI**	***p*-value**	**Pooled HR**	**95% CI**	***p* value**
Model 1						
Hypophosphatemia	0.46	0.27 – 0.79	.005	0.67	0.52 – 0.86	.001
Normophosphatemia	1.00 (ref)	-	-	1.00 (ref)	-	-
Hyperphosphatemia	46.33	38.54 – 55.69	<.0001	4.40	3.79 – 5.10	<.0001
Model 2						
Hypophosphatemia	0.43	0.25 – 0.74	.002	0.71	0.55 – 0.91	.007
Normophosphatemia	1.00 (ref)	-	-	1.00 (ref)	-	-
Hyperphosphatemia	44.84	37.28 – 53.93	<.0001	5.97	5.12 – 6.95	<.0001
Model 3						
Hypophosphatemia	2.07	1.19 – 3.60	.009	0.89	0.69 – 1.15	.37
Normophosphatemia	1.00 (ref)	-	-	1.00 (ref)	-	-
Hyperphosphatemia	2.46	1.96 – 3.09	<.0001	2.99	2.47 – 3.63	<.0001
Model 4						
Hypophosphatemia	2.17	1.24 – 3.78	.007	0.88	0.69 – 1.15	.36
Normophosphatemia	1.00 (ref)	-	-	1.00 (ref)	-	-
Hyperphosphatemia	2.59	2.05 – 3.27	<.0001	3.14	2.58 – 3.82	<.0001
	Number of events: 477			Number of events: 1050		

Model 1: unadjusted analysis.

Model 2: adjusted for age and sex.

Model 3: model 2 plus time-updated eGFR and proteinuria.

Model 4: model 3 plus donor age, donor sex and donor status, cold and warm ischemia time, number of HLA mismatches, primary renal disease, CMV infection of the recipient, antihypertensive drug use, systolic blood pressure, plasma corrected calcium, dialysis vintage, decade of transplantation, immunosuppressive drug use, delayed graft function.

Abbreviations: CI, confidence interval; DCGF, death-censored graft failure; HR, hazard ratio.

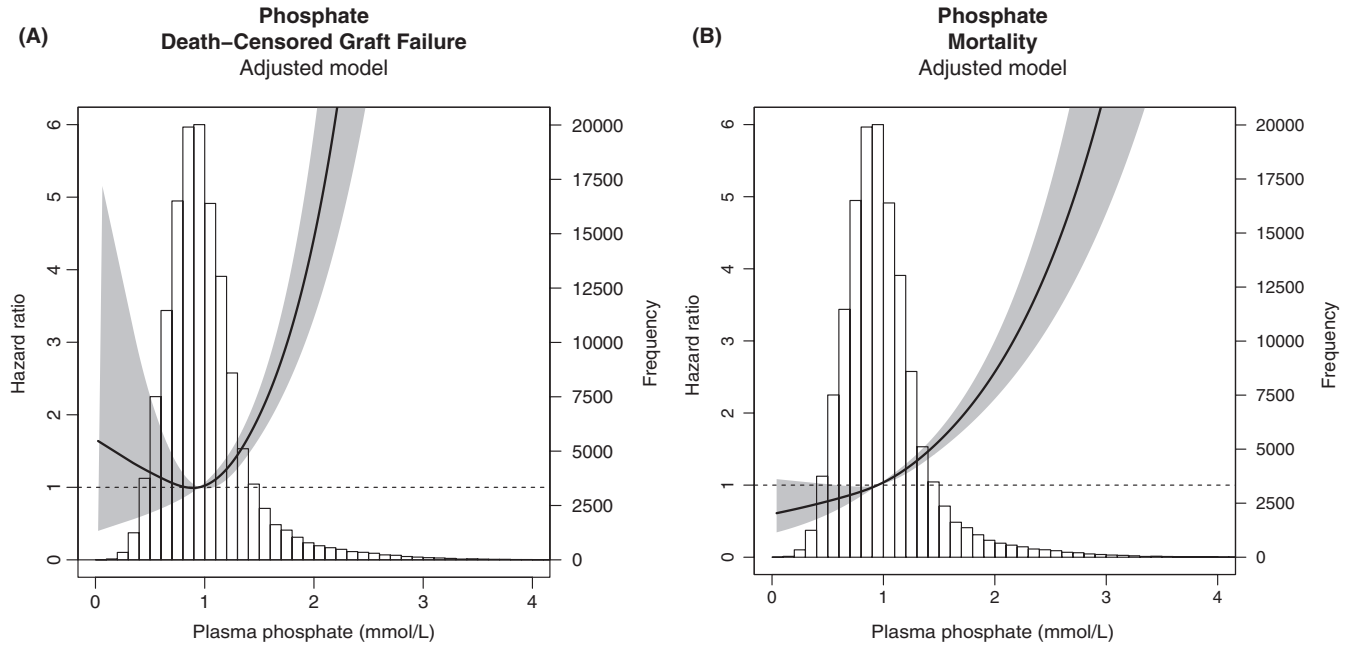

FIGURE 4 Spline curves illustrating the association between plasma phosphate levels and the risk of (A) death-censored graft failure and (B) all-cause mortality. Models are on the basis of a cubic spline term (restricted cubic spline) with three knots. The solid lines represent the fully adjusted hazard ratios (HRs) for death-censored graft failure (Cox regression model 4) and all-cause mortality (Cox regression model 4). The gray areas represent the 95% confidence intervals of the HRs

epithelium, contributing to the risk of graft failure,[36,37] and in the vascular wall leading to vascular calcification.[38] We found that plasma phosphate levels above the upper limit of normal are associated with an increased risk of all-cause mortality. In a previous study we found that posttransplant hypophosphatemia, based on the lowest intraindividual plasma phosphate level, was associated with a lower DCGF risk.[6] Surprisingly, in the current analyses, using all available phosphate data in a time-updated analysis with a median follow-up of 16 years and upon adjustment for time-updated eGFR, hypophosphatemia was significantly associated with an increased risk of DCGF, compared to normophosphatemia. Although this result could not be confirmed in sensitivity analyses and therefore should be interpreted with caution, it could be speculated that hypophosphatemia triggered phosphate supplementation, in turn promoting nephrocalcinosis.[39]

Several treatment strategies are available to correct plasma calcium and phosphate levels. Cessation of vitamin D supplementation may resolve hypercalcemia, although elevated plasma calcium may persist without vitamin D supplements particularly in patients with hyperparathyroidism. The calcimimetic cinacalcet may correct both hypercalcemia and hypophosphatemia in kidney transplant recipients.[40] Moreover, a randomized controlled trial comparing cinacalcet and parathyroidectomy showed that parathyroidectomy was superior in achieving normocalcemia, reducing PTH levels, and increasing bone mineral density.[41] Further research should focus on comparing available treatment options with clinical outcome measures such as mortality, graft function, and patient's quality of life.

Some limitations of our study should be addressed. No data were available on calcium and phosphate supplementation after transplantation, nor could we include data on the use of phosphate binders, vitamin D supplements, or other medication interfering with calcium/phosphate homeostasis. Next, despite adjusting Cox regression models for a variety of potential confounders, we cannot exclude residual confounding, for example by plasma vitamin D or FGF-23 which were not routinely measured. Generalizability of our findings might be compromised due to our predominantly Caucasian patient population. A major strength of the current study is that we used a large dataset including several time-updated variables, enhancing statistical power. Also, our findings were robust upon sensitivity analyses in several subgroups of our cohort, including subsets of patients with pretransplant PTH levels available, excluding extreme calcium or phosphate levels, and censoring values with simultaneous eGFR <45 mL/min/1.73 m.^{2} To our knowledge, this is the largest study with long-term follow-up investigating the relationship between posttransplant plasma calcium and phosphate levels and outcomes adjusting for time-updated variables.

In conclusion, in this observational cohort study of kidney transplant recipients, posttransplant hypercalcemia, even in the presence of preserved kidney function, was associated with an increased mortality risk while associations of hyperphosphatemia with DCGF and mortality may be driven by eGFR. Further research should prospectively evaluate strategies to normalize calcium-phosphate homeostasis following kidney transplantation.

ACKNOWLEDGMENTS

S.J.L.B. is principal investigator of the Transplant Lines Biobank and Data Repository of the University Medical Center Groningen.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

AUTHOR CONTRIBUTIONS

M.H.d.B. and W.Y.v.d.P. designed the study; W.Y.v.d.P and A.W.G.N. analyzed the data; W.Y.v.d.P., A.W.G.N., S.P.B., R.A.P., S.K., S.J.L.B., and M.H.d.B. contributed to the interpretation of data and drafted and revised the paper; all the authors approved the final version of the manuscript.

ORCID

Willemijn Y. van der Plas https://orcid.org/0000-0002-5758-2959
Stefan P. Berger https://orcid.org/0000-0003-2228-4676
Robert A. Pol https://orcid.org/0000-0002-2236-5233
Schelto Kruijff https://orcid.org/0000-0002-5151-7320
Stephan J. L. Bakker https://orcid.org/0000-0003-3356-6791
Martin H. de Borst https://orcid.org/0000-0002-4127-8733

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Plas WY, Gomes Neto AW, Berger SP, et al. Association of time-updated plasma calcium and phosphate with graft and patient outcomes after kidney transplantation. *Am J Transplant*. 2021;21:2437–2447. https://doi.org/10.1111/ajt.16457