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

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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 ethical committee
MICE	multiple imputation by chained equations
n	number
PRD	primary renal disease
PTH	parathyroid hormone
SD	standard deviation
UMCG	University Medical Center Groningen

Abstract

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 2,769 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. Post-transplant hypercalcemia was associated with an increased risk of mortality (1.63 [1.31–2.00], P<0.0001), but not DCGF. Hyperphosphatemia was associated with both DCGF (2.59 [2.05–3.27], P<0.0001) and mortality (3.14 [2.58-3.82], P<0.0001). 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.

Post-transplant 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.

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.

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 pre-existing 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 three months after transplantation.[6] Post-transplant hypophosphatemia is likely caused by inappropriately high PTH and fibroblast-growth factor (FGF)-23 combined with recovered kidney function in the early post-transplant stage, driving phosphaturia.[7] On the other hand, hyperphosphatemia may also occur, particularly with impaired graft function.

The prognostic implications of post-transplant 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 data set 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]

Materials and Methods

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/1.73m^2) or during intensive care unit admission not taken into account. This study was approved by the local medical ethical 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.

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 of 2006 were performed on the Merck Mega Analyzer (Merck, Darmstadt, Germany); measurements after this date were performed on the Roche Modular (Roche Ltd., Mannheim, Germany). Laboratory measurements prior to March 2006 were converted according to equations listed in Supplemental Table 1. The PTH assay used in our hospital changed in 2006 from Nichols Institute Diagnostics (San Juan Capistrano, CA, USA) to Immulite 2500 (Siemens Healthcare Diagnostics, Deerfield, IL, USA) and Cobas e601 immunology analyzer (Roche Diagnostics, Mannheim, Germany). 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.

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]

Study endpoints

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

Statistical analyses

Continuous variables are reported as mean ± standard deviation (SD) for normally distributed variables or median with interquartile range (IQR) for non-normally 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 pre-transplant PTH value closest to the transplantation date was included in sub-analyses. 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 post-transplantation. 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 3 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 re-run with the

imputed dataset after excluding the 0.5% highest and lowest calcium and phosphate values. Third, we assessed whether pre-transplant PTH affected the associations of plasma calcium and phosphate levels with the risk of DCGF and mortality in a subgroup with pre-transplant 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 one year post-transplant. Finally, we censored plasma calcium and phosphate data at any timepoint when the simultaneously measured eGFR was <45 mL/min/1.73m^2; the calcium and phosphate value of the previous measurement with a simultaneous eGFR ≥45 mL/min/1.73m^2 was carried forward. Patients who never reached an eGFR ≥45 mL/min/1.73m^2 were excluded from this analysis.

Statistical analysis was performed using SPSS Statistics version 25.0 (IBM Corporation, Armonk, NY, USA) and R version 3.2.6 (Vienna, Austria); a P-value of <0.05 was considered statistically significant.

Results

Baseline characteristics

We included 2,769 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 post-mortal donors (71.5%); 537 (19.4%) patients underwent a pre-emptive transplantation. Pre-transplant PTH levels were available in 1,412 patients (51%). Median pre-transplant 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 2–9 times the upper limit of normal. Median follow-up duration was 16.3 (8.7–25.2) years.

Plasma calcium and phosphate over time

Overall, 138,496 plasma calcium and phosphate measurements were available from 2,769 patients, with a median of 43 (31–61) measurements per patient. The intra-individual mean plasma corrected calcium concentration was 2.39±0.18 mmol/L (9.56±0.05 mg/dL). Within the first year post-transplant, 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 intra-individual 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).

Post-transplant plasma calcium, phosphate and the risk of DCGF

During 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 (Supplemental Table 2), adjusting for PTH in a subgroup with PTH-levels available

(Supplemental Table 4), after excluding 0.5% highest and lowest plasma calcium levels (Supplemental Table 6), and in a subgroup with patients with eGFR ≥45 mL/min/1.73 m^2 at one-year post-transplant (Supplemental Table 8).

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=0.007). The association between hypophosphatemia and DCGF persisted in a sensitivity analysis after excluding the 0.5% highest and lowest plasma phosphate values (Supplementary Table 7), however could not be reproduced when analyzing the original (pre-imputation) data, or in subgroups of patients with available pre-transplant PTH levels or patients with eGFR ≥45 mL/min/1.73 m^2 at one-year post-transplant (Supplemental Tables 3, 5, and 9, 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<0.0001), 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 (Supplemental Table 3), in patients with PTH-measurements available (Supplemental Table 5), after exclusion of the 0.5% highest and lowest plasma phosphate levels (Supplemental Table 7), and in patients with an eGFR ≥45 mL/min/1.73 m^2 at 1-year post-transplantation (Supplemental Table 9). 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 (Supplemental Table 11).

Post-transplant 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<0.0001), 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<0.0001, Table 2 and Figure 3). The association between hypercalcemia and mortality persisted in all sensitivity analyses (Supplemental Tables 2, 4, 6, 8). 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 (Supplemental Table 10).

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=0.36), as shown in Table 3 and Figure 4. Hyperphosphatemia was associated with a 3-fold risk of death, compared to normophosphatemia (fully adjusted HR 3.14 [95% CI 2.58–3.82], p<0.001). Similar findings were observed in sensitivity analyses (Supplemental Tables 3, 5, 7, 9). Upon censoring all calcium 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=0.07; Supplemental Table 11).

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 (Supplemental Table 12) and phosphate (Supplemental Table 13).

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 post-transplant 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]

Several factors may contribute to the disturbances in mineral metabolism observed after kidney transplantation. In the early post-transplant 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 two 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 post-transplant. 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 post-transplant patients.[5] On top of this, already low pre-transplant 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 post-transplant 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.[30] 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.[31–34] We did not observe a significant relationship between hypocalcemia and clinical outcomes in this study.

Several previous studies have demonstrated associations of plasma phosphate with an increased risk of (death-censored) graft failure and all-cause mortality.[10,11,35–37] Mechanistically, hyperphosphatemia, particularly when combined with higher calcium levels, may promote calcium-phosphate crystal deposition in the tubular epithelium, contributing to the risk of graft failure,[37,38] and in the vascular wall leading to vascular calcification.[39] 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 post-transplant hypophosphatemia, based on the lowest intra-individual 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.[40]

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 hyperphosphatemia in kidney transplant recipients.[41] 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.[42] 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 pre-transplant 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 post-transplant plasma calcium and phosphate levels and outcomes adjusting for time-updated variables.

In conclusion, in this observational cohort study of kidney transplant recipients, post-transplant 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.

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 authors approved the final version of the manuscript.

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Disclosure

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

Figure Legends

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

Figure 2 – Distribution of proportion of patients with a monthly mean plasma calcium (panel A) or plasma phosphate (panel B) within, below or above the reference range for the first 12 months (upper panel) and during long-term follow-up (lower panel)

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.

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.

References

1. Slinin Y, Foley RN, Collins AJ: Calcium, phosphorus, parathyroid hormone, and cardiovascular disease in hemodialysis patients: The USRDS waves 1, 3, and 4 study. *J. Am. Soc. Nephrol.* 16: 1788–1793, 2005

2. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM: Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J. Am. Soc. Nephrol.* [Internet] 15: 2208–18, 2004

3. Tentori F, Blayney MJ, Albert JM, Gillespie BW, Kerr PG, Bommer J, et al.: Mortality Risk for Dialysis Patients With Different Levels of Serum Calcium, Phosphorus, and PTH: The Dialysis Outcomes and Practice Patterns Study (DOPPS). *Am. J. Kidney Dis.* [Internet] 52: 519–530, 2008

4. Reinhardt W, Bartelworth H, Jockenhövel F, Schmidt-Gayk H, Witzke O, Wagner K, et al.: Sequential changes of biochemical bone parameters after kidney transplantation. *Nephrol. Dial. Transplant* [Internet] 13: 436–42, 1998

5. Evenepoel P, Van Den Bergh B, Naesens M, De Jonge H, Bammens B, Claes K, et al.: Calcium metabolism in the early posttransplantation period. *Clin. J. Am. Soc. Nephrol.* 4: 665–672, 2009

6. van Londen M, Aarts BM, Deetman PE, van der Weijden J, Eisenga MF, Navis G, et al.: Post-Transplant Hypophosphatemia and the Risk of Death-Censored Graft Failure and Mortality after Kidney Transplantation. *Clin. J. Am. Soc. Nephrol.* [Internet] 12: 1301–1310, 2017

7. Evenepoel P, Naesens M, Claes K, Kuypers D, Vanrenterghem Y: Tertiary "hyperphosphatoninism" accentuates hypophosphatemia and suppresses calcitriol levels in renal transplant recipients. *Am. J. Transplant.* 7: 1193–1200, 2007

8. Özdemir FN, Afsar B, Akgul A, Usluoğullari C, Akçay A, Haberal M: Persistent hypercalcemia is a significant risk factor for graft dysfunction in renal transplantation recipients. In: *Transplantation Proceedings*, pp 480–482, 2006

9. Boom H, Mallat MJK, De Fijter JW, Paul LC, Bruijn JA, Van Es LA: Calcium levels as a risk factor for delayed graft function. *Transplantation* 77: 868–873, 2004

10. Moore J, Tomson CRV, Tessa Savage M, Borrows R, Ferro CJ: Serum phosphate and calcium concentrations are associated with reduced patient survival following kidney transplantation. *Clin. Transplant.* [Internet] 25: 406–416, 2011

11. Benavente D, Chue CD, Moore J, Addison C, Borrows R, Ferro CJ: Serum phosphate measured at 6 and 12 months after successful kidney transplant is independently associated with subsequent graft loss. *Exp. Clin. Transplant.* 10: 119–124, 2012

12. Eisenga MF, Gomes-Neto AW, Van Londen M, Ziengs AL, Douwes RM, Stam SP, et al.: Rationale and design of TransplantLines: A prospective cohort study and biobank of solid organ transplant recipients. *BMJ Open* 8: 2018

13. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, et al.: A new equation to estimate glomerular filtration rate. *Ann. Intern. Med.* 150: 604–612, 2009

14. Boxma PY, van den Berg E, Geleijnse JM, Laverman GD, Schurgers LJ, Vermeer C, et al.: Vitamin K Intake and Plasma Desphospho-Uncarboxylated Matrix Gla-Protein Levels in Kidney Transplant Recipients. *PLoS One* 7: 2012

15. Venkat-Raman G, Tomson CRV, Gao Y, Cornet R, Stengel B, Gronhagen-Riska C, et al.: New primary renal diagnosis codes for the ERA-EDTA. *Nephrol. Dial. Transplant.* 27: 4414–4419, 2012

16. Little RJA, Rubin DB: Statistical analysis with missing data.

17. Fine JP, Gray RJ: A Proportional Hazards Model for the Subdistribution of a Competing Risk. *J. Am. Stat. Assoc.* 94: 496, 1999

18. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group: KDIGO clinical practice guideline for the diagnosis, evaluation, prevention and treatment of chronic kidney disease mineral and bone disorder (CKD-MBD). *Kidney Int Suppl* [Internet] 7: 1–59, 2017

19. Cundy T, Kanis JA, Heynen G, Morris PJ, Oliver DO: Calcium metabolism and hyperparathyroidism after renal transplantation. *Q. J. Med.* [Internet] 52: 67–78, 1983

20. Messa P, Vallone C, Mioni G, Geatti O, Turrin D, Passoni N, et al.: Direct in vivo assessment of parathyroid hormone-calcium relationship curve in renal patients. In: *Kidney International*, pp 1713–1720, 1994

21. Lou I, Foley D, Odorico SK, Leverson G, Schneider DF, Sippel R, et al.: How Well Does Renal Transplantation Cure Hyperparathyroidism? *Ann. Surg.* [Internet] 262: 653–9, 2015

22. Borchhardt K, Sulzbacher I, Benesch T, Födinger M, Sunder-Plassmann G, Haas M: Low-Turnover Bone Disease in Hypercalcemic Hyperparathyroidism After Kidney Transplantation. *Am. J. Transplant.* [Internet] 7: 2515–2521, 2007

23. Vangala C, Pan J, Cotton RT, Ramanathan V: Mineral and bone disorders after kidney transplantation. Front. Med. 5: 2018

24. Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, et al.: Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: Results of the study to evaluate early kidney disease. *Kidney Int.* [Internet] 71: 31–38, 2007

25. Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, et al.: FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J. Bone Miner. Res.* 19: 429–435, 2004

26. Levi M: Post-transplant hypophosphatemia. *Kidney Int.* 59: 2377–2387, 2001

27. Schankel K, Robinson J, Bloom RD, Guerra C, Rader D, Joffe M, et al.: Determinants of coronary artery calcification progression in renal transplant recipients. *Am. J. Transplant.* 7: 2158–2164, 2007

28. Naganuma T, Takemoto Y, Uchida J, Nakatani T, Kabata D, Shintani A: Hypercalcemia Is a Risk Factor for the Progression of Aortic Calcification in Kidney Transplant Recipients. *Kidney Blood Press. Res.* 44: 823–834, 2019

29. Mazzaferro S, Pasquali M, Taggi F, Baldinelli M, Conte C, Muci ML, et al.: Progression of coronary artery calcification in renal transplantation and the role of secondary hyperparathyroidism and inflammation. *Clin. J. Am. Soc. Nephrol.* 4: 685–690, 2009

30. Moore J, Tomson CRV, Tessa Savage M, Borrows R, Ferro CJ: Serum phosphate and calcium concentrations are associated

with reduced patient survival following kidney transplantation. *Clin. Transplant.* [Internet] 25: 406–416, 2011

31. Roden DM, Lazzara R, Rosen M, Schwartz PJ, Towbin J, Vincent GM: Multiple mechanisms in the long-QT syndrome. Current knowledge, gaps, and future directions. The SADS Foundation Task Force on LQTS. *Circulation* [Internet] 94: 1996–2012, 1996

32. Elming H, Holm E, Jun L, Torp-Pedersen C, Køber L, Kircshoff M, et al.: The prognostic value of the QT interval and QT interval dispersion in all-cause and cardiac mortality and morbidity in a population of Danish citizens. *Eur. Heart J.* [Internet] 19: 1391–400, 1998

33. Obi Y, Park C, Soohoo M, Sumida K, Hamano T, Rhee CM, et al.: Association of Pre-ESRD Serum Calcium With Post-ESRD Mortality Among Incident ESRD Patients: A Cohort Study. *J. Bone Miner. Res.* [Internet] 33: 1027–1036, 2018

34. Borrego-Utiel FJ, Pérez-del Barrio MPM de. P, Biechy-Baldan M del M, Segura-Torres P: Cinacalcet may prolong the QT interval in patients on haemodialysis with secondary hyperparathyroidism. *Nefrologia* 33: 272–273, 2013

35. Schaeffner ES, Födinger M, Kramar R, Sunder-Plassmann G, Winkelmayer WC: Prognostic associations of serum calcium, phosphate and calcium phosphate concentration product with outcomes in kidney transplant recipients. *Transpl. Int.* 20: 247–255, 2007

36. Merhi B, Shireman T, Carpenter MA, Kusek JW, Jacques P, Pfeffer M, et al.: Serum Phosphorus and Risk of Cardiovascular Disease, All-Cause Mortality, or Graft Failure in Kidney Transplant Recipients: An Ancillary Study of the FAVORIT Trial Cohort. *Am. J. Kidney Dis.* 70: 377–385, 2017

37. Sampaio MS, Molnar MZ, Kovesdy CP, Mehrotra R, Mucsi I, Sim JJ, et al.: Association of pretransplant serum phosphorus with posttransplant outcomes. *Clin. J. Am. Soc. Nephrol.* 6: 2712–2721, 2011

38. Evenepoel P, Lerut E, Naesens M, Bammens B, Claes K, Kuypers D, et al.: Localization, etiology and impact of calcium phosphate deposits in renal allografts. *Am. J. Transplant.* 9: 2470–2478, 2009

39. London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H: Arterial media calcification in end-stage renal disease: Impact on all-cause and cardiovascular mortality. *Nephrol. Dial. Transplant.* 18: 1731–1740, 2003

40. Baia LC, Heilberg IP, Navis G, De Borst MH: Phosphate and FGF-23 homeostasis after kidney transplantation [Internet]. Nat. Rev. Nephrol. 11: 656–666, 2015

41. Evenepoel P, Cooper K, Holdaas H, Messa P, Mourad G, Olgaard K, et al.: A Randomized Study Evaluating Cinacalcet to Treat Hypercalcemia in Renal Transplant Recipients With Persistent Hyperparathyroidism. *Am. J. Transplant.* [Internet] 14: 2545–2555, 2014

42. Cruzado JM, Moreno P, Torregrosa J V., Taco O, Mast R, Gomez-Vaquero C, et al.: A Randomized Study Comparing Parathyroidectomy with Cinacalcet for Treating Hypercalcemia in Kidney Allograft Recipients with Hyperparathyroidism. *J. Am. Soc. Nephrol.* [Internet] 27: 2487–2494, 2016

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Tables

Table 1

Baseline characteristics of the cohort

	Total population
	N = 2769
Patient characteristics	
Female sex, n (%)	1171 (42.3)
Age, yrs	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)*
Pre-emptive 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, hrs	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. BMI, body mass index; ESRD, end-stage renal disease; CMV, cytomegalovirus; HLA, human leukocyte antigen.

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	<0.0001	1.64	1.39 – 1.93	<0.0001
	Normocalcemia	1.00 (ref)	-	-	1.00 (ref)	-	-
	Hypercalcemia	0.49	0.29 – 0.84	0.009	1.72	0.40 – 2.11	<0.0001
Model 2	Hypocalcemia	4.53	3.73 – 5.50	<0.0001	1.78	1.51 – 2.09	<0.0001
	Normocalcemia	1.00 (ref)	-	-	1.00 (ref)	-	-
	Hypercalcemia	0.54	00.32 – 0.93	0.03	1.49	1.21 – 1.84	0.0002
Model 3	Hypocalcemia	1.17	0.95 – 1.14	0.33	1.32	1.12 – 1.57	0.001
	Normocalcemia	1.00 (ref)	-	-	1.00 (ref)	-	-
	Hypercalcemia	0.67	0.38 – 1.17	0.16	1.51	1.22 – 1.59	0.0001
Model 4	Hypocalcemia	1.02	0.82 – 1.27	0.79	1.09	0.91 – 1.36	0.32
	Normocalcemia	1.00 (ref)	-	-	1.00 (ref)	-	-
	Hypercalcemia	0.77	0.44 – 1.35	0.71	1.63	1.31 – 2.00	<0.0001
		Number of events: 477			Number of events: 1050		

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

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.

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	0.005	0.67	0.52 – 0.86	0.001
	Normophosphatemia	1.00 (ref)	-	-	1.00 (ref)	-	-
	Hyperphosphatemia	46.33	38.54 – 55.69	<0.0001	4.40	3.79 – 5.10	<0.0001
Model 2	Hypophosphatemia	0.43	0.25 – 0.74	0.002	0.71	0.55 – 0.91	0.007
	Normophosphatemia	1.00 (ref)	-	-	1.00 (ref)	-	-
	Hyperphosphatemia	44.84	37.28 – 53.93	<0.0001	5.97	5.12 – 6.95	<0.0001
Model 3	Hypophosphatemia	2.07	1.19 – 3.60	0.009	0.89	0.69 – 1.15	0.37
	Normophosphatemia	1.00 (ref)	-	-	1.00 (ref)	-	-
	Hyperphosphatemia	2.46	1.96 – 3.09	<0.0001	2.99	2.47 – 3.63	<0.0001
Model 4	Hypophosphatemia	2.17	1.24 – 3.78	0.007	0.88	0.69 – 1.15	0.36
	Normophosphatemia	1.00 (ref)	-	-	1.00 (ref)	-	-
	Hyperphosphatemia	2.59	2.05 – 3.27	<0.0001	3.14	2.58 – 3.82	<0.0001
		Number of events: 477			Number of events: 1050		

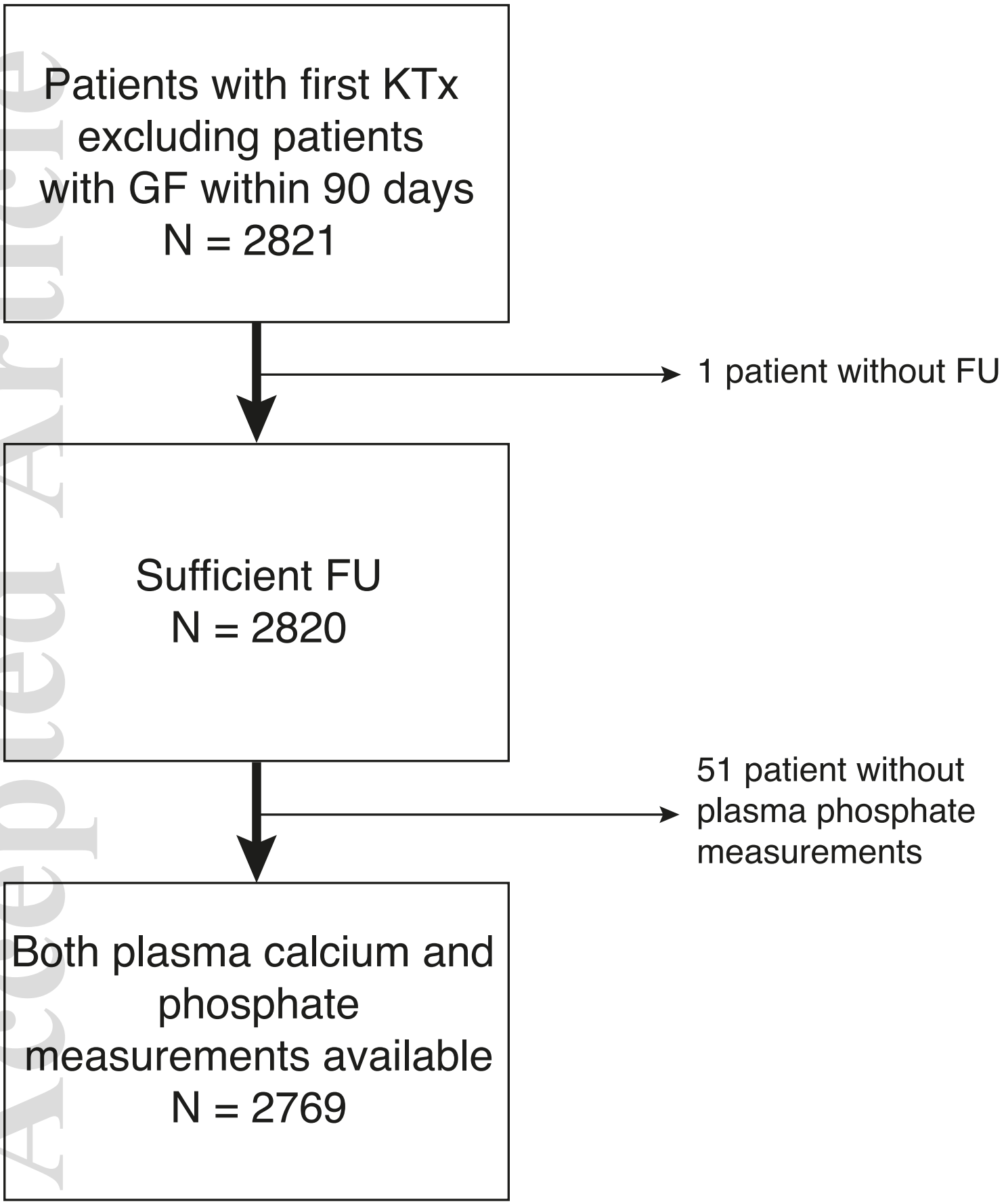
DCGF, death-censored graft failure; HR, hazard ratio; CI, confidence interval.

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.



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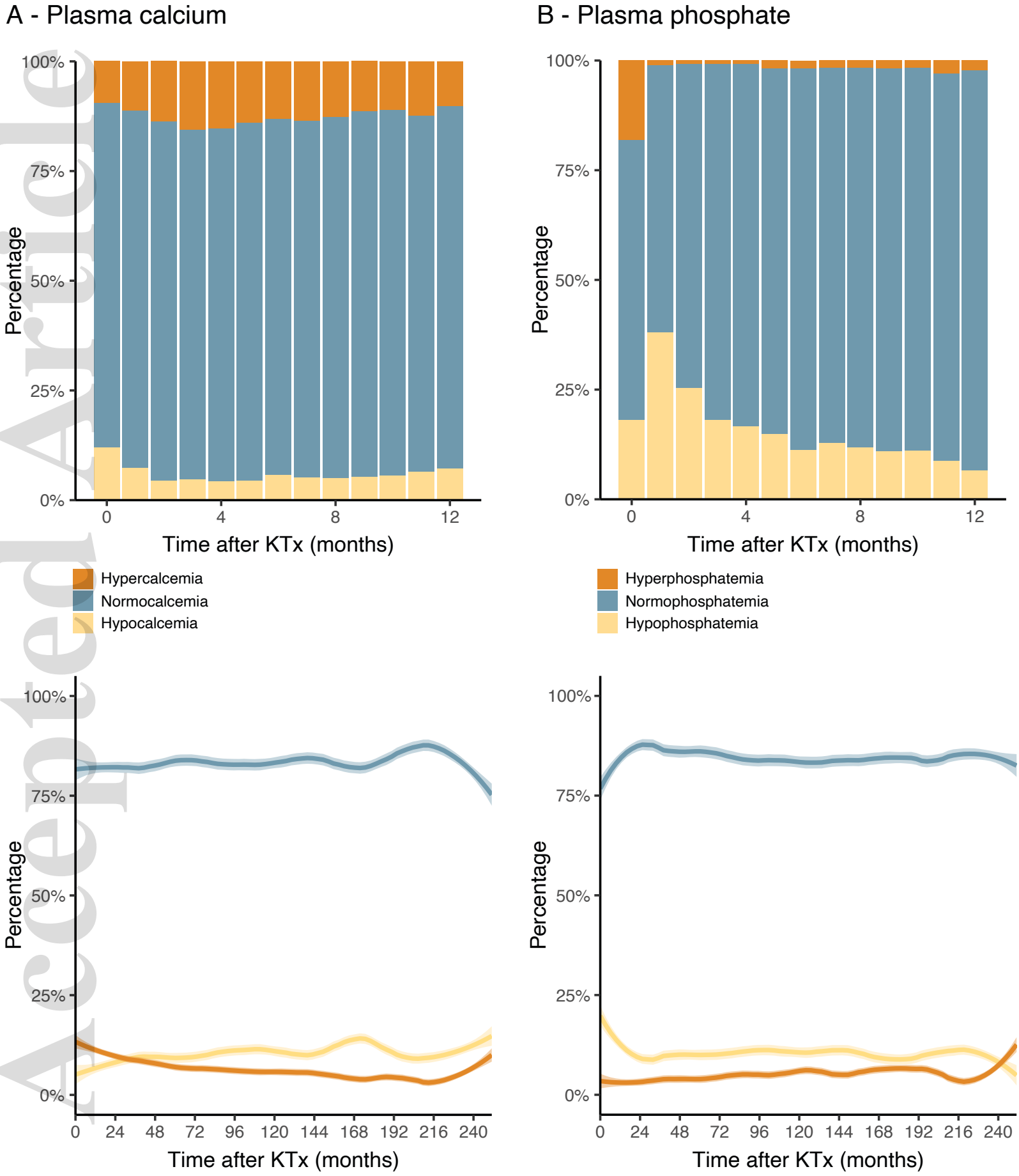

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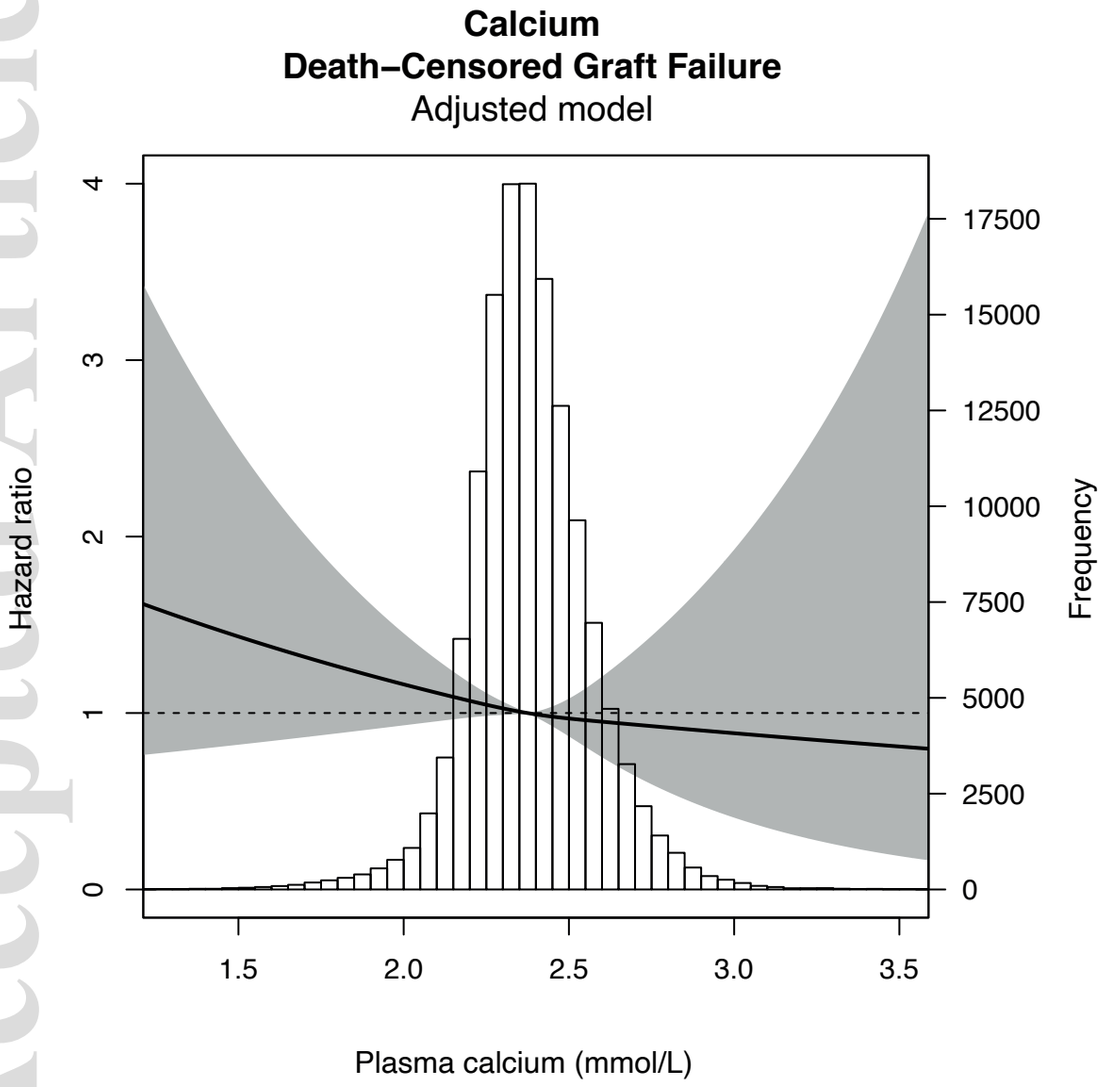

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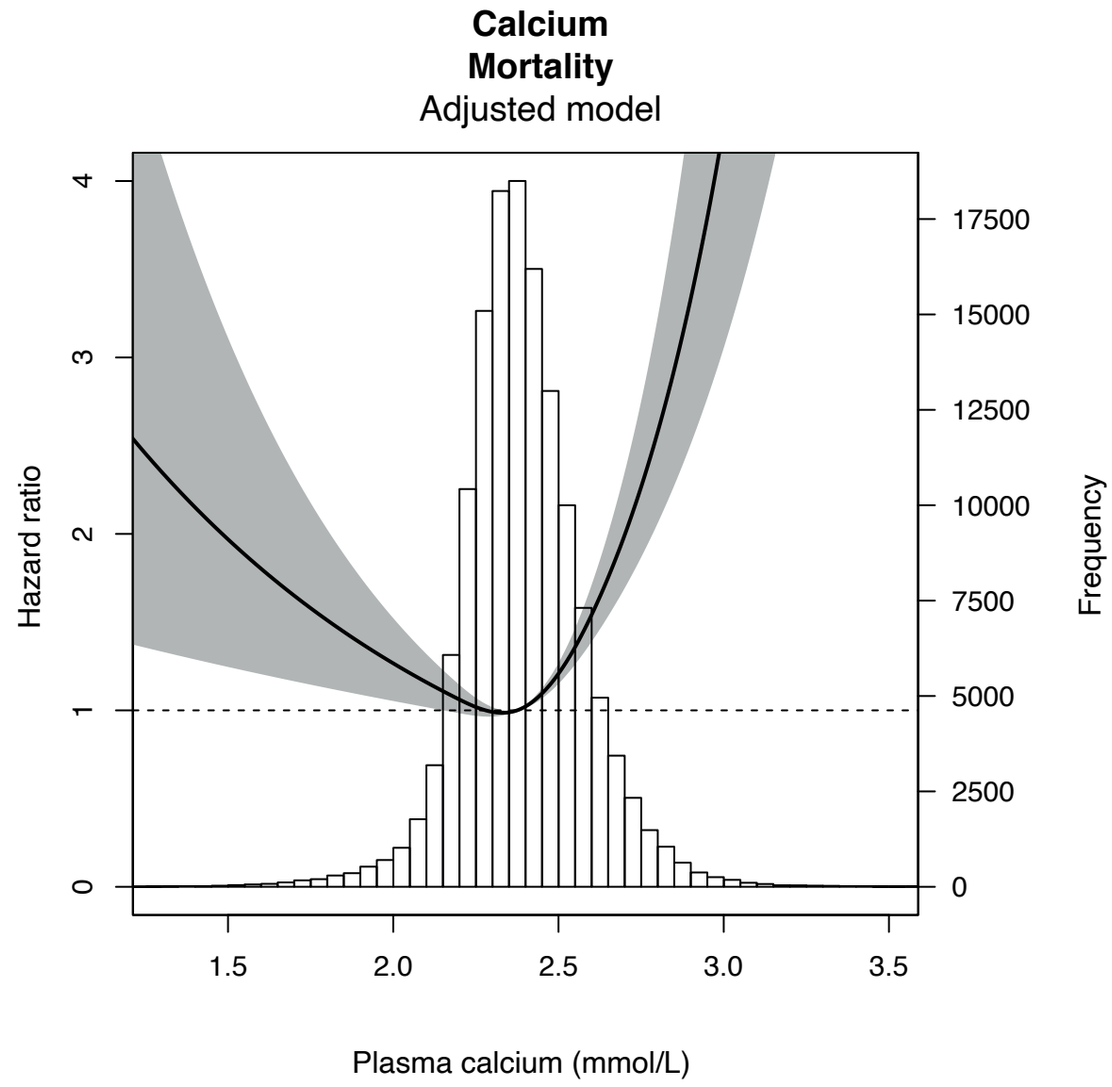

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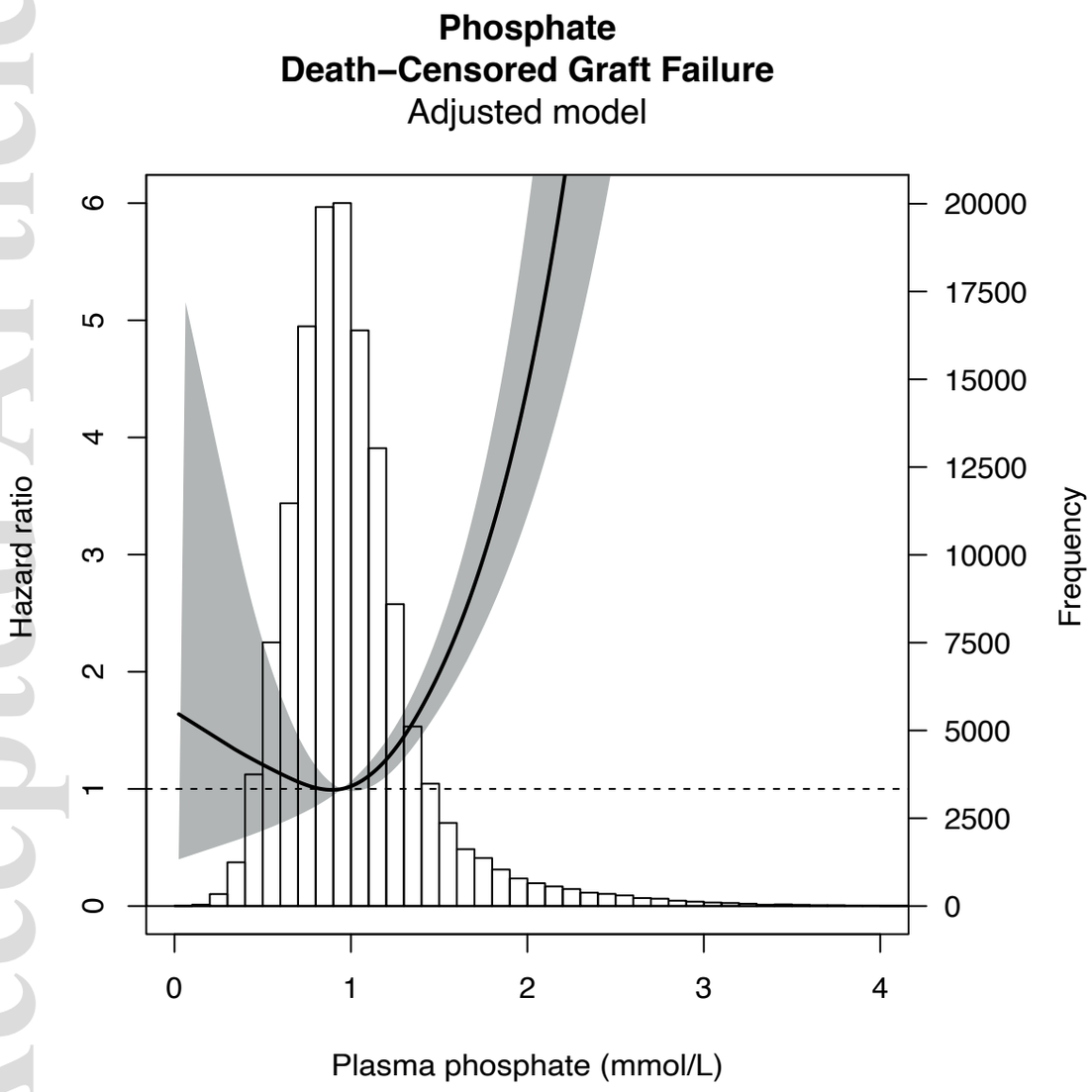

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