Living kidney donor evaluation and safety assessment
van Londen, Marco

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CHAPTER 7

Post-transplant Hypophosphatemia and the Risk of Death-censored Graft Failure and Mortality after Kidney Transplantation

Marco van Londen, MD*, Brigitte M. Aarts, MSc*, Petronella E. Deetman, MD, PhD, Jessica van der Weijden, BSc, Michele F. Eisenga, MD, Gerjan Navis, MD, PhD, Stephan J.L. Bakker, MD, PhD and Martin H. de Borst, MD, PhD, on behalf of the NIGRAM consortium


Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen and University of Groningen, Groningen, the Netherlands

* Contributed equally
Abstract

Background and objectives: Hypophosphatemia is common in the first year after kidney transplantation, but its clinical implications are unclear. We investigated the relationship between the severity of post-transplant hypophosphatemia and mortality or death-censored graft failure in a large cohort of renal transplant recipients with long-term follow-up.

Design, setting, participants, and measurements: We performed a longitudinal cohort study in 957 renal transplant recipients who were transplanted between 1993 and 2008 at a single center. We used a large real-life dataset containing 28,178 phosphate measurements (27 [23-34] (median [first-third quartile]) serial measurements per patient), and selected the lowest intra-individual phosphate level during the first year after transplantation. The primary outcomes were all-cause mortality, cardiovascular mortality, and death-censored graft failure.

Results: The median [IQR] intra-individual lowest phosphate level was 1.58 [1.30-1.95] mg/dL, and was reached at 33 [21-51] days post-transplant. Estimated GFR (CKD-EPI) was the main correlate of the lowest serum phosphate level ($R^2=0.32$). During 9 [5-12] years of follow-up, 181 (19%) patients developed graft failure and 295 (35%) patients died, of which 94 (32%) due to cardiovascular disease. More severe hypophosphatemia was associated with a lower risk of death-censored graft failure (final model hazard ratio [95% confidence interval] per 1 mg/dL 1.64 [1.14-2.34]) and cardiovascular mortality (2.72 [1.61-4.61]), but not non-cardiovascular mortality (0.75 [0.51-1.11]) or all-cause mortality (0.87 [0.62-1.23]). Multivariable adjustment for potential confounders did not materially change the results.

Conclusions: Post-transplant hypophosphatemia develops early after transplantation. These data connect post-transplant hypophosphatemia with favorable long-term graft and patient outcomes.
Introduction

Chronic kidney disease (CKD) is characterized by progressively deregulated phosphate homeostasis, reflected by increased circulating levels of the phosphaturic hormones fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) and hyperphosphatemia. Emerging data have shown strong positive associations between plasma FGF23 concentrations and adverse cardiovascular outcomes, and FGF23 may contribute to the development of left ventricular hypertrophy in CKD. After kidney transplantation, FGF23 and PTH concentrations decline over a period of weeks to months. In the early post-transplant stage, the relatively high FGF23 and PTH concentrations in the context of a restored renal excretory capacity of phosphate may result in hypophosphatemia, which usually persists for weeks to months.

The clinical implications of post-transplant hypophosphatemia in terms of graft and patient survival are unclear. On the one hand, hypophosphatemia may reflect good graft function and thereby be associated with prolonged graft survival and favorable cardiovascular outcomes. On the other hand, post-transplant hypophosphatemia may reflect long-term exposure to high circulating FGF23 and PTH concentrations before transplantation, which may at least in part persist after transplantation. Such high FGF23 and PTH levels might contribute to an increased risk of graft failure and cardiovascular complications.

We investigated whether the development of post-transplant hypophosphatemia and the lowest serum phosphate concentration in the first year after transplantation are associated with death-censored graft failure, as well as cardiovascular and all-cause mortality in a large cohort of renal transplant recipients.

Materials and Methods

Study population

This single-center cohort consisted of 957 renal transplant recipients who underwent a first (solo) kidney transplantation and had a functioning graft at one year after transplantation. All transplantsations were performed at the University Medical Center Groningen, the Netherlands, or in a collaborating center in The Netherlands on behalf of the Dutch donor exchange program. From 1,288 kidney transplantations performed between January 1993 and February 2008, 151 patients were excluded because of re-transplantation, 65 because of simultaneous kidney/liver or kidney/pancreas transplantation, 5 because of missing serum phosphate data, and 4 were lost to follow-up. Finally, we excluded 106 patients with...
graft survival <1 year. After excluding these patients, 957 patients remained within the study cohort. The study was approved by the institutional ethical review board (METc 2014/077). All procedures were conducted in accordance with the declarations of Helsinki and Istanbul.

Transplantation and follow-up
Standard immunosuppression consisted of triple therapy with tacrolimus (Prograft® or Avagraf®, Astellas Pharma b.v. Leiden, the Netherlands; initial trough level 8-12 ng/mL, followed by 6-10 ng/mL (>2 months) and 4-6 ng/mL (>6 months) or cyclosporine microemulsion (Neoral®, Novartis Pharma b.v. Arnhem, the Netherlands; 2 times 4 mg/kg daily; initial trough level 200-500 ug/L, 150-500 ug/L (>2 months) and 75-125 ug/L (>6 months), in combination with mycophenolate mofetil (Cellcept®, Roche b.v. Woerden, the Netherlands; 2g daily or Myfortic®, Novartis Pharma, b.v. Arnhem, the Netherlands; 1440 mg daily), and prednisolone. Patients were followed up in our center, initially weekly, tapered to 4-6 weekly during the first year, and later with longer intervals up to once per year. Phosphate concentrations were assessed at each visit. Management of calcium/phosphate metabolism was not subject to a formal treatment protocol during the timeframe of this study. Oral phosphate supplementation was given as potassium phosphate/sodium phosphate for symptomatic or asymptomatic severe hypophosphatemia, at the discretion of the treating nephrologist. Data about phosphate and vitamin D supplementation and parathyroidectomy was collected before transplantation and before the occurrence of the lowest serum phosphate.

Study outcomes
The primary outcomes of this study were death-censored graft failure (defined as end stage renal disease requiring dialysis or re-transplantation), all-cause mortality, and cardiovascular mortality. The cause of death was obtained from death certificates coded by a physician, according to the International Classification of Diseases, Ninth Revision (ICD-9), and were verified by reviewing the medical records. Cardiovascular mortality was defined as death from acute myocardial infarction (ICD-9 code 410), acute and subacute ischemic heart disease (ICD-9 code 411), coronary artery bypass grafting (ICD-9 code 414) or percutaneous transluminal coronary angioplasty, subarachnoid hemorrhage (ICD-9 code 430), intracerebral hemorrhage (ICD-9 code 431), other intracranial hemorrhage (ICD-9 code 432), occlusion or stenosis of the precerebral (ICD-9 code 433) or cerebral (ICD-9 code 434) arteries, or other vascular interventions such as percutaneous transluminal angioplasty or bypass grafting of aorta and peripheral vessels.
Clinical and biochemical data
Data from all routinely determined biochemical measurements from all patients (real-life dataset) within the cohort were used. All routine measurements before March 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). From 1993 until 2006, PTH analysis was performed on indication using the PTH-intact assay from Nichols Institute Diagnostics (San Juan Capistrano, CA, USA). Since February 2006, PTH has been analyzed using PTH-intact assays using an Immulite 2500 (Siemens Healthcare Diagnostics, Deerfield, IL, USA) and a Cobas e601 immunochemistry analyzer (Roche Diagnostics, Mannheim, Germany). For the current study, measurements obtained before March 2006 were converted according to the conversion equations that are provided in Supplemental Table S1. The lowest available serum phosphate concentration during the first year after kidney transplantation for each patient was used in the analyses. Post-transplant hypophosphatemia was categorized as severe (lowest serum phosphate <1.55 mg/dL (<0.5 mmol/L), mild hypophosphatemia (lowest serum phosphate 1.55-2.17 mg/dL (0.5-0.7 mmol/L)) or absent (>2.17 mg/dL (>0.7 mmol/L)), according to the local reference value. Phosphate concentrations at fixed timepoints 6 and 12 months after transplantation were also collected. Renal function was estimated using the creatinine-based CKD-EPI formula, using serum creatinine obtained on the same day as the lowest serum phosphate. Other biochemical parameters were collected on the same day as the lowest serum phosphate (±2 weeks). Clinical and transplant-related data were collected from our central hospital registry. Post-transplant diabetes mellitus (PTDM) was defined according to the American Diabetes Association guidelines: casual plasma glucose ≥200 mg/dL (≥11.1 mmol/L) or fasting plasma ≥126 mg/dL (≥ 7.0 mmol/L) or use of anti-diabetic medication. Rejection assessment was based on histological analysis by a pathologist and graded according to the BANFF classification.

Statistical analyses
Variable distribution was tested using histograms and probability plots. Normally distributed variables are presented as mean ± standard deviation, and non-normally distributed variables as median [first – third quartile], unless indicated otherwise. Non-normally distributed variables were log-transformed. Differences between hypophosphatemia categories were tested using one-way ANOVA for normally distributed variables, Kruskal-Wallis test for non-normally distributed variables and Pearson’s χ² test for nominal variables (α=0.05).
To identify independent correlates of the lowest serum phosphate, backward linear regression was used with the following covariates: recipient age, sex, smoking status, etiology of kidney disease (glomerular disease, glomerulonephritis, tubular-interstitial disease, polycystic kidney disease, dysplasia, renovascular disease, diabetes and other),
Chapter 7

CMV status, donor type, cold ischemia time, warm ischemia time, acute rejection, delayed graft function, proteinuria, PTH, calcium, albumin, estimated GFR, 24h urea excretion, immunosuppressive use (calcineurin-inhibitor, mTOR inhibitor, proliferation inhibitor, corticosteroids, other), HLA A/B/DR mismatches, systolic blood pressure, diastolic blood pressure, pre-transplant diabetes, pre-transplant cardiovascular event, BMI, donor CMV status, donor age and donor sex. Variables that were significantly correlated in the final backward regression model were considered independent correlates of the lowest serum phosphate.

To investigate the association of hypophosphatemia severity with the study outcomes, univariable and multivariable Cox regression analyses were performed. Hypophosphatemia was analyzed as either a categorical (‘absent’ vs. ‘mild’ and absent vs. ‘severe’) or a continuous variable (per 1 mg/dL). Model 1 was adjusted for recipient age and sex, which are important potential confounders in many associations. In model 2, we added eGFR given its potential influence on both the lowest serum phosphate and the risk of adverse graft and patient outcomes. Model 3 was additionaly adjusted for non-immunological transplant-related factors (donor age and sex, donor type, cold ischemia time, delayed graft function, proteinuria at time of lowest serum phosphate), model 4 was adjusted for immunological transplant-related factors (total number of HLA mismatches, acute rejection, induction and maintenance immunosuppressive use, and panel of reactive antibodies), and model 5 was adjusted for potential confounders related to cardiovascular outcomes (dialysis vintage, smoking status, pre-transplant diabetes mellitus, and CV history). Finally, model 6 was adjusted for additional factors that might influence serum phosphate levels and patient or graft outcomes: phosphate and vitamin D supplementation, corrected calcium concentrations, and parathyroidectomy. To visualize the hazard ratio for patients with hypophosphatemia, spline curves were made using the fully adjusted Cox regression models for all outcomes, based a cubic spline term (restricted cubic spline) with 3 knots (0.51;1.59;5.79), with a serum phosphate level of 2.17 mg/dL (lower limit of normal phosphate) as reference value.

Additional covariates as reported in the Results were added to multivariable model 6 unless specified otherwise. We tested for effect modifications by invoking multiplicative interaction terms for eGFR and timing of the lowest serum phosphate. We also tested for hazard proportionality over time. Based on missing data analysis (Supplemental Table S2), we used listwise deletion for our analyses, and used imputed data in additional analyses. Due to a relatively high number of unknown causes of death, we investigated whether these missing data could have influenced our model in an exploratory analysis, by labeling all unknown causes of death as cardiovascular.
Post-transplant hypophosphatemia and clinical outcomes

Results

Patient characteristics and correlates of post-transplant hypophosphatemia
Baseline characteristics according to categories of hypophosphatemia (absent, mild or severe) are shown in Table 1. A total number of 28,178 phosphate measurements were analyzed, with a median [first-third quartile] of 27 [23-34] phosphate measurements per patient during the first year post-transplant. Patients who developed severe hypophosphatemia (<1.55 mg/dL, n=446, 47%) had a higher eGFR, were more often male, were less likely to have delayed graft function and had shorter ischemia times, compared with patients who did not develop hypophosphatemia. The lowest serum phosphate concentration was reached at 33 [21-51] days after transplantation. The time from transplantation to lowest serum phosphate was similar among hypophosphatemia categories. The intra-individual lowest serum phosphate concentrations were significantly lower than serum phosphate concentrations measured at 6 or 12 months post-transplantation (P<0.001, Figure 1). Multivariable linear regression analysis revealed eGFR at the time of lowest phosphate as independent correlate of the lowest serum phosphate (-0.14 mg/dL per 10 ml/min/1.73m², [95% CI -0.24 to -0.04], P=0.007, model R²=0.32).

Post-transplant hypophosphatemia and graft failure.
During a median follow-up of 9 [5-12] years after transplantation, 181 (19%) patients developed graft failure. Compared with no hypophosphatemia, both mild and severe hypophosphatemia were associated with a lower risk of death-censored graft failure. Also when analyzing the lowest phosphate as a continuous variable, a more severe hypophosphatemia was associated with a lower risk of death-censored graft failure (final model HR per 1 mg/dL 1.64 [95% CI 1.14-2.34]). This association remained significant after adjustment for potential confounders (Figure 2, Table 2). We found significant effect modification by eGFR (Pinteraction<0.001). Upon stratification, the association between hypophosphatemia category and death-censored graft failure was only significant in patients with an eGFR<40 mL/min/1.73 m² (Figure 3). There was no effect modification by the timing of the lowest serum phosphate (Pinteraction =0.19).

A sensitivity analysis (Supplemental Table S2) indicated that missing data are not a cause of bias in our analyses, but in an additional analysis we used multiple imputation to handle missing data. Additional adjustment for serum PTH at the time of lowest phosphate in a
Table 1. Clinical characteristics of the cohort

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=957)</th>
<th>Absent hypophosphatemia (n=136)</th>
<th>Mild hypophosphatemia (n=375)</th>
<th>Severe hypophosphatemia (n=446)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest serum phosphate post-transplant, mg/dL</td>
<td>1.58 [1.30-1.95]</td>
<td>2.45 [2.26-2.73]</td>
<td>1.80 [1.67-1.98]</td>
<td>1.24 [1.05-1.42]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, n (%) male</td>
<td>557 (58 %)</td>
<td>64 (47%)</td>
<td>221 (59%)</td>
<td>272 (61%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Smoking status, n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>48 (5%)</td>
<td>5 (4%)</td>
<td>13 (4%)</td>
<td>30 (7%)</td>
<td>0.40</td>
</tr>
<tr>
<td>No smoking</td>
<td>488 (51%)</td>
<td>65 (48%)</td>
<td>188 (50%)</td>
<td>235 (52%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>421 (44%)</td>
<td>66 (49%)</td>
<td>174 (46%)</td>
<td>181 (41%)</td>
<td></td>
</tr>
<tr>
<td>Etiology of kidney disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary glomerular disease</td>
<td>24.6 %</td>
<td>26.8%</td>
<td>24.9 %</td>
<td>20.9 %</td>
<td>0.44</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>5.5 %</td>
<td>4.6 %</td>
<td>6.8 %</td>
<td>6.2 %</td>
<td></td>
</tr>
<tr>
<td>Tubulo-interstitial nephritis / pyelonephritis</td>
<td>10.2 %</td>
<td>10.8%</td>
<td>9.0 %</td>
<td>14.0 %</td>
<td></td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>18.4 %</td>
<td>20.2 %</td>
<td>18.4 %</td>
<td>16.3 %</td>
<td></td>
</tr>
<tr>
<td>Renovascular</td>
<td>8.5 %</td>
<td>9.2 %</td>
<td>8.8 %</td>
<td>7.0 %</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>4.1 %</td>
<td>3.7 %</td>
<td>4.7 %</td>
<td>4.7 %</td>
<td></td>
</tr>
<tr>
<td>Dysplasia</td>
<td>1.8 %</td>
<td>1.4 %</td>
<td>2.5 %</td>
<td>1.6 %</td>
<td></td>
</tr>
<tr>
<td>Other/unknown</td>
<td>24.1 %</td>
<td>23.4 %</td>
<td>24.9 %</td>
<td>29.5 %</td>
<td></td>
</tr>
<tr>
<td>Panel of Reactive Antibodies, %</td>
<td>8.0 %</td>
<td>8.0 %</td>
<td>9.0 %</td>
<td>10.0 %</td>
<td>0.54</td>
</tr>
<tr>
<td>Donor type living/cadaveric, n</td>
<td>220/737</td>
<td>28/108</td>
<td>64/311</td>
<td>128/318</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Warm ischaemia time, min</td>
<td>40 [33-50]</td>
<td>40 [35-52]</td>
<td>40 [33-51]</td>
<td>39 [32-45]</td>
<td>0.02</td>
</tr>
<tr>
<td>Delayed graft function, n (%)</td>
<td>270 (28%)</td>
<td>50 (37%)</td>
<td>110 (29%)</td>
<td>110 (25%)</td>
<td>0.02</td>
</tr>
<tr>
<td>sympitive, n (%)</td>
<td>31 (3.2%)</td>
<td>8 (5.9%)</td>
<td>11 (2.9%)</td>
<td>12 (2.7%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum calcium pre-Tx, mg/dL</td>
<td>9.62 (1.04)</td>
<td>9.62 (1.12)</td>
<td>9.70 (1.12)</td>
<td>9.94 (0.92)</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum PTH pre-Tx, pg/mL</td>
<td>160.3 [72.4-393.7]</td>
<td>103.7 [61.1-212.2]</td>
<td>122.6 [57.3-396.1]</td>
<td>188.6 [88.2-393.7]</td>
<td>0.04</td>
</tr>
<tr>
<td>Acute rejection, n (%)</td>
<td>342 (36%)</td>
<td>47 (35%)</td>
<td>135 (36%)</td>
<td>160 (36%)</td>
<td>0.95</td>
</tr>
<tr>
<td>Estimated GFR (CKD-EPI)*, mL/min/1.73 m²</td>
<td>52 [39-66]</td>
<td>41 [26-54]</td>
<td>49 [38-60]</td>
<td>58 [46-70]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphate supplementation, n (%)</td>
<td>67 (7.0%)</td>
<td>7 (5.2%)</td>
<td>18 (4.8%)</td>
<td>42 (9.4%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Vitamin D supplementation, n (%)</td>
<td>176 (18.4%)</td>
<td>41 (30.1%)</td>
<td>57 (15.2%)</td>
<td>78 (17.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin D supplementation pre-Tx, n (%)</td>
<td>399 (41.7%)</td>
<td>47 (34.6%)</td>
<td>149 (39.7%)</td>
<td>203 (45.5%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Post-transplantation parathyroidectomy, n (%)</td>
<td>75 (7.8%)</td>
<td>11 (8.1%)</td>
<td>25 (6.7%)</td>
<td>39 (8.7%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Hypophosphatemia symptoms**, n(%)</td>
<td>5 (0.5%)</td>
<td>1 (0.74%)</td>
<td>3 (0.8%)</td>
<td>1 (0.22%)</td>
<td>0.44</td>
</tr>
</tbody>
</table>
### Post-Transplant Hypophosphatemia and Clinical Outcomes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium*, mg/dL</td>
<td>9.22 (0.96)</td>
<td>9.06 (1.04)</td>
<td>9.38 (0.96)</td>
<td>9.46 (0.96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Corrected calcium*, mg/dL</td>
<td>9.57 (0.82)</td>
<td>9.32 (0.89)</td>
<td>9.55 (0.78)</td>
<td>9.67 (0.83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum PTH*, pg/mL</td>
<td>85.8 [61.5-137.8]</td>
<td>69.5 [47.4-132.8]</td>
<td>85.8 [70.0-129.5]</td>
<td>91.3 [87.3-140.3]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proteinuria*, g/24h</td>
<td>0.3 [0.2-0.6]</td>
<td>0.2 [0.3-0.6]</td>
<td>0.4 [0.2-0.7]</td>
<td>0.2 [0.3-0.6]</td>
<td>0.30</td>
</tr>
<tr>
<td>Albumin*, g/dL</td>
<td>3.8 [3.4-4.1]</td>
<td>3.6 [3.4-4.0]</td>
<td>3.8 [3.4-4.2]</td>
<td>3.8 [3.4-4.1]</td>
<td>0.65</td>
</tr>
<tr>
<td>24h urea excretion*, mg/dL</td>
<td>2673.22 [2100.38-3302.21]</td>
<td>2511.44 [2131.27-3108.46]</td>
<td>2701.29 [2091.58-3341.52]</td>
<td>2673.22 [2091.96-3324.67]</td>
<td>0.22</td>
</tr>
</tbody>
</table>

**Medication use**

- **Cyclosporine use**
  - Group 1: 87.9 %
  - Group 2: 93.4 %
  - Group 3: 87.7 %
  - Group 4: 86.3 %
  - p-value: 0.09

- **Cyclosporine trough*, mg/l**
  - Group 1: 224 (165)
  - Group 2: 209 (125)
  - Group 3: 223 (144)
  - Group 4: 230 (190)
  - p-value: 0.16

- **Tacrolimus use**
  - Group 1: 7.4 %
  - Group 2: 2.9 %
  - Group 3: 7.5 %
  - Group 4: 8.7 %
  - p-value: 0.08

- **Tacrolimus trough*, mg/l**
  - Group 1: 11.1 (6.0)
  - Group 2: 8.1 (4.9)
  - Group 3: 11.6 (5.9)
  - Group 4: 11.4 (6.2)
  - p-value: 0.56

- **Proliferation inhibitor**
  - Group 1: 76.8 %
  - Group 2: 80.9 %
  - Group 3: 76.8 %
  - Group 4: 75.6 %
  - p-value: 0.46

- **mTOR inhibitor**
  - Group 1: 3.8 %
  - Group 2: 1.5 %
  - Group 3: 4.3 %
  - Group 4: 4.0 %
  - p-value: 0.31

- **Corticosteroids**
  - Group 1: 96.4 %
  - Group 2: 98.5 %
  - Group 3: 97.3 %
  - Group 4: 95.1 %
  - p-value: 0.08

- **Other**
  - Group 1: 23.4 %
  - Group 2: 26.5 %
  - Group 3: 24.3 %
  - Group 4: 21.7 %
  - p-value: 0.46

**Induction therapy**

- **Anti-thymocyte globulin, n (%)**
  - Group 1: 87 (9 %)
  - Group 2: 10 (7 %)
  - Group 3: 38 (10 %)
  - Group 4: 39 (9 %)
  - p-value: 0.59

- **Anti-IL2 MoAb, n (%)**
  - Group 1: 410 (43 %)
  - Group 2: 62 (45 %)
  - Group 3: 152 (41 %)
  - Group 4: 196 (44 %)
  - p-value: 0.48

- **Other/unknown, n (%)**
  - Group 1: 460 (48 %)
  - Group 2: 64 (47 %)
  - Group 3: 185 (49 %)
  - Group 4: 211 (47 %)
  - p-value: 0.82

- **CMV positive status, n (%)**
  - Group 1: 376 (39.5 %)
  - Group 2: 180 (40.5 %)
  - Group 3: 141 (37.6 %)
  - Group 4: 180 (40.5 %)
  - p-value: 0.74

- **Pre-transplantation diabetes mellitus (no/DM1/DM2), %**
  - Group 1: 94/24/2
  - Group 2: 93/2/5
  - Group 3: 94/3/3
  - Group 4: 94/24/2
  - p-value: 0.82

- **BMI at transplantation, kg/m²**
  - Group 1: 24.3 [22.0-27.2]
  - Group 2: 23.7 [21.9-28.3]
  - Group 3: 24.6 [22.0-27.1]
  - Group 4: 24.3 [22.0-27.2]
  - p-value: 0.94

- **Pre-Tx CV-event, n (%)**
  - Group 1: 111 (11.9 %)
  - Group 2: 9 (6.9 %)
  - Group 3: 46 (12.8 %)
  - Group 4: 56 (12.6 %)
  - p-value: 0.16

- **First systolic blood pressure after Tx , mmHg**
  - Group 1: 140 [130-152]
  - Group 2: 140 [131-150]
  - Group 3: 140 [130-155]
  - Group 4: 140 [133-150]
  - p-value: 0.92

- **First diastolic blood pressure after Tx , mmHg**
  - Group 1: 84 [79-90]
  - Group 2: 82 [79-90]
  - Group 3: 85 [80-90]
  - Group 4: 84 [78-90]
  - p-value: 0.65

**Donor characteristics**

- **Age, y**
  - Group 1: 47 [35-55]
  - Group 2: 47 [38-54]
  - Group 3: 47 [35-56]
  - Group 4: 46 [33-54]
  - p-value: 0.65

- **Sex, n (%) male**
  - Group 1: 477 (49.9 %)
  - Group 2: 66 (48.5 %)
  - Group 3: 197 (52.5 %)
  - Group 4: 214 (48 %)
  - p-value: 0.41

- **CMV positive status, n (%)**
  - Group 1: 475 (49.9 %)
  - Group 2: 76 (57.1 %)
  - Group 3: 170 (45.3 %)
  - Group 4: 229 (51.6 %)
  - p-value: 0.12

**Mismatched HLA**

- **A (0/1/2), valid %**
  - Group 1: 47/46/7
  - Group 2: 43/48/9
  - Group 3: 49/45/6
  - Group 4: 45/47/8
  - p-value: 0.35

- **B (0/1/2), valid %**
  - Group 1: 40/47/1
  - Group 2: 37/51/13
  - Group 3: 43/44/14
  - Group 4: 37/50/13
  - p-value: 0.53

- **DR (0/1/2), valid %**
  - Group 1: 52/45/2
  - Group 2: 54/44/2
  - Group 3: 56/42/2
  - Group 4: 48/48/3
  - p-value: 0.12

* Measured at the time of lowest serum phosphate level

** Symptoms attributed to hypophosphatemia by treating nephrologist
Figure 1. Lowest serum phosphate and serum phosphate concentrations at 6 and 12 months after transplantation, displayed as median and interquartile range for (A) severe, (B) mild and (C) absent hypophosphatemia. The overall lowest serum phosphate concentrations were significantly different from serum phosphate measured at 6 or 12 months ($P<0.001$ in all categories).

subgroup of patients (final model HR per 1 mg/dL 1.65 [1.13-2.42]), number of phosphate measurements (final model HR 1.62 [1.11-2.34]) or the time between transplantation and lowest serum phosphate (final model HR 1.69 [95% CI 1.17-2.40]) did not change the results, nor did replacing eGFR by creatinine clearance (final model HR 1.64 [95% CI 1.15-2.34]).

Post-transplant hypophosphatemia and mortality
During follow-up 295 (35%) patients died, of which 94 (32%) died from cardiovascular causes, 41 (14%) of infectious causes, 40 (14%) of malignancies and 13 (4%) of other causes. The cause of death was unknown in 107 patients (36%). The development of hypophosphatemia after transplantation was not associated with all-cause mortality (Table
Figure 2. Spline curve illustrating the association between the lowest serum phosphate levels and the risk of (A) graft failure, (B) all-cause mortality, (C) cardiovascular mortality and (D) non-cardiovascular mortality. Models are based on a cubic spline term (restricted cubic spline) with 3 knots (0.51; 1.59; 5.79), with a serum phosphate level of 1.58 mg/dL as reference value. The solid line represents the fully adjusted hazard ratio (HR) for graft failure (Cox regression model 6), all-cause and cardiovascular mortality (Cox regression model 6). The grey area represents the 95% CI of the HR.

However, severe post-transplant hypophosphatemia was associated with a lower risk of cardiovascular mortality compared with no hypophosphatemia (final model HR 0.37 [95% CI 0.17-0.81]; continuous HR per 1 mg/dL 2.72 [95% CI 1.61-4.61]). When the regression model was built in a non-cumulative method to reduce the number of adjustments for the number of events, the results were materially similar in all models (results not shown). The lowest serum phosphate was not associated with death by infectious disease (n=41, HR 0.91 [95% CI 0.49-1.68]), malignancies (n=40, HR 0.84 [95% CI 0.44-1.62]), other causes (n=13, HR 0.87 [95% CI 0.46-1.63]) or unknown causes (n=107, HR 0.92 [95% CI 0.61-1.40]).
Table 2. Cox regression model of serum phosphate and graft failure

<table>
<thead>
<tr>
<th>Hypophosphatemia category</th>
<th>Absent</th>
<th>Mild</th>
<th>Severe</th>
<th>Lowest serum phosphate per 1 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft failure (181 events)</td>
<td></td>
<td></td>
<td></td>
<td>HR [95% CI] P value</td>
</tr>
<tr>
<td>Crude</td>
<td>0.52 [0.36-0.77]**</td>
<td>0.44 [0.30-0.64]***</td>
<td>2.31 [1.79-3.00] &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.55 [0.38-0.82]**</td>
<td>0.46 [0.31-0.68]***</td>
<td>2.19 [1.69-2.84] &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>0.63 [0.42-0.92]**</td>
<td>0.61 [0.41-0.91]*</td>
<td>1.94 [1.47-2.56] &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>0.82 [0.51-1.34]</td>
<td>0.72 [0.43-1.19]</td>
<td>1.78 [1.27-2.49] 0.001</td>
<td></td>
</tr>
<tr>
<td>Model 4</td>
<td>0.87 [0.51-1.48]</td>
<td>0.72 [0.42-1.26]</td>
<td>1.67 [1.18-2.36] 0.004</td>
<td></td>
</tr>
<tr>
<td>Model 5</td>
<td>0.79 [0.46-1.38]</td>
<td>0.74 [0.42-1.29]</td>
<td>1.54 [1.09-2.19] 0.02</td>
<td></td>
</tr>
<tr>
<td>Model 6</td>
<td>0.79 [0.45-1.38]</td>
<td>0.71 [0.40-1.25]</td>
<td>1.64 [1.14-2.34] 0.007</td>
<td></td>
</tr>
</tbody>
</table>

Model 1: adjusted for recipient age and sex
Model 2: model 1 + eGFR at time of lowest phosphate
Model 3: model 2 + donor age and sex, donor type, cold ischemia time, delayed graft function, proteinuria (at time of lowest serum phosphate)
Model 4: model 3 + total number of HLA mismatches, acute rejection, induction and maintenance immunosuppressive use, panel of reactive antibodies
Model 5: model 4 + cardiovascular risk factors (dialysis vintage, smoking status, pre-transplant diabetes mellitus, CV history)
Model 6: model 5 + corrected calcium concentrations (before transplantation and at time of lowest serum phosphate)

* P<0.05; ** P<0.01; *** P<0.001

Figure 3
The risk of graft failure in crude Cox regression analysis, according to eGFR above or below 40 mL/min/1.73 m². (A) In patients with an eGFR <40 mL/min/1.73 m², mild (hazard ratio (HR) 0.44 [95% confidence interval (CI) 0.26-0.75], P=0.003) and severe (HR 0.38 [95% CI 0.20-0.71], P=0.002) hypophosphatemia were significantly associated with the risk of graft failure, compared to absent hypophosphatemia (70 events in this stratum). (B) In patients with eGFR ≥40 mL/min/1.73 m² no significant association was observed, both for mild (HR 0.93 [95% CI 0.48-1.81], P=0.84) and severe (HR 0.85 [95% CI 0.44-1.62], P=0.62) hypophosphatemia, compared to absent hypophosphatemia (109 events in this stratum).
Table 3. Cox regression model of serum phosphate and mortality

<table>
<thead>
<tr>
<th>Hypophosphatemia category</th>
<th>Lowest serum phosphate per 1 mg/dL</th>
<th>All-cause mortality (295 events)</th>
<th>CV mortality (94 events)</th>
<th>Non-CV mortality (176 events)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR [95% CI]</td>
<td>P value</td>
<td>HR [95% CI]</td>
<td>P value</td>
</tr>
<tr>
<td>Absent</td>
<td>1.01 [0.69-1.47]</td>
<td>0.97 [0.67-1.41]</td>
<td>0.73 [0.42-1.29]</td>
<td>0.58 [0.33-1.02]</td>
</tr>
<tr>
<td>Mild</td>
<td></td>
<td></td>
<td>0.65 [0.37-1.16]</td>
<td>0.53 [0.30-0.94]</td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td>0.65 [0.37-1.15]</td>
<td>0.53 [0.29-0.97]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.64 [0.35-1.18]</td>
<td>0.50 [0.26-0.95]</td>
</tr>
<tr>
<td>Crude</td>
<td>Ref</td>
<td></td>
<td>0.65 [0.37-1.16]</td>
<td>0.53 [0.30-0.94]*</td>
</tr>
<tr>
<td>Model 1</td>
<td>Ref</td>
<td></td>
<td>0.65 [0.37-1.15]</td>
<td>0.53 [0.29-0.97]*</td>
</tr>
<tr>
<td>Model 2</td>
<td>Ref</td>
<td></td>
<td>0.64 [0.35-1.18]</td>
<td>0.50 [0.26-0.95]*</td>
</tr>
<tr>
<td>Model 3</td>
<td>Ref</td>
<td></td>
<td>0.62 [0.32-1.18]</td>
<td>0.44 [0.22-0.88]*</td>
</tr>
<tr>
<td>Model 4</td>
<td>Ref</td>
<td></td>
<td>0.44 [0.21-0.91]*</td>
<td>0.36 [0.17-0.77]**</td>
</tr>
<tr>
<td>Model 5</td>
<td>Ref</td>
<td></td>
<td>0.44 [0.21-0.91]*</td>
<td>0.36 [0.17-0.77]**</td>
</tr>
<tr>
<td>Model 6</td>
<td>Ref</td>
<td></td>
<td>0.45 [0.21-0.95]*</td>
<td>0.37 [0.17-0.81]*</td>
</tr>
</tbody>
</table>

Model 1: adjusted for recipient age and sex
Model 2: model 1 + eGFR at time of lowest phosphate
Model 3: model 2 + donor age and sex, donor type, cold ischemia time, delayed graft function, proteinuria (at time of lowest serum phosphate)
Model 4: model 3 + total number of HLA mismatches, acute rejection, induction and maintenance immunosuppressive use, panel of reactive antibodies
Model 5: model 4 + cardiovascular risk factors (dialysis vintage, smoking status, pre-transplant diabetes mellitus, CV history)
Model 6: model 5 + corrected calcium concentrations (before transplantation and at time of lowest serum phosphate)

* P<0.05; ** P<0.01; *** P<0.001
When assuming that all patients with unknown cause of death died due to cardiovascular disease, results were similar (final model HR per 1 mg/dL 2.04 [95% CI 1.72-2.41]). Additional adjustment for serum PTH at the time of lowest phosphate in a subgroup (final model HR 2.88 [1.70-4.91]), number of phosphate measurements (final model HR 2.00 [95% CI 1.72-2.40]), time between transplantation and lowest serum phosphate (final model HR 2.13[95%CI 1.80-2.52]) or replacing eGFR with creatinine clearance (final model HR 1.92 [95% CI 1.63-2.26]) did not materially change the association between hypophosphatemia and cardiovascular mortality. No significant interaction with eGFR or time after transplantation was observed.

**Discussion**

Hypophosphatemia is very common after kidney transplantation: in our study, the incidence of hypophosphatemia (serum phosphate <2.17 mg/dL) was 86%. Moreover, when analyzing the clinical implications of post-transplant hypophosphatemia, we found that the occurrence of hypophosphatemia was associated with favorable graft and patient outcomes. We analyzed the lowest serum phosphate concentration reached within the first year after transplantation, considering a large number of intra-individual phosphate measurements (N >28,000 real-life measurements for the full cohort). We found that patients who developed hypophosphatemia had a lower risk of graft failure compared to those who did not develop hypophosphatemia. Furthermore, post-transplant hypophosphatemia was associated with a lower risk of cardiovascular mortality, but not all-cause mortality.

In line with our results, previous studies found that a lower serum phosphate concentration at 6 and 12 months after transplantation was associated with a lower risk of graft failure. These fixed time points may have been relatively late; given our finding that 50% of patients develop their lowest serum phosphate concentration at approximately one month after transplantation. When considering all serum phosphate measurements performed within the first year after transplantation, and selecting the lowest serum phosphate value at any given moment in the first year, we found that the incidence of severe hypophosphatemia was 47% in our cohort, which is in line with some²³, but not all²⁴, previous studies.

The development of hypophosphatemia was associated with a higher eGFR, which is in line with the concept that good graft function is accompanied by an increased renal capacity to excrete phosphate. Our findings do not support a previously postulated hypothesis that hypophosphatemia would contribute to nephrocalcinosis and premature graft failure²⁵. We hypothesize that hypophosphatemia is indicative of a restored renal capacity to filtrate phosphate to provide a tubular response to the high concentrations of phosphaturic
hormones FGF23 and PTH, by means of reducing sodium-phosphate co-transporters 2a and 2c (NaPi-2a/2c). We cannot distinguish whether hypophosphatemia is a mere marker of a better prognosis, or whether lower phosphate concentrations may actually protect graft function. For clinical purposes, our data raise the question whether the associations of hypophosphatemia with favorable graft and patient outcomes should have consequences for phosphate management after transplantation. Yet, proof for an etiological role of low phosphate levels should precede clinical recommendations.

Interestingly, we found significant interaction by eGFR for the association between the severity of hypophosphatemia and the risk of death-censored graft failure. Patients with a lower eGFR (<40 mL/min/1.73 m²), but a preserved capacity to excrete sufficient amounts of phosphate to induce hypophosphatemia, apparently had better graft outcome than those with a low eGFR and no hypophosphatemia. In patients with an eGFR ≥40 mL/min/1.73 m², the risk of developing death-censored graft failure was much compared to patients with an eGFR <40 mL/min/1.73 m². Interestingly, the association between hypophosphatemia and graft survival remained materially unchanged after adjustment for eGFR, suggesting that factors other than the eGFR are also involved. Only 21% of the variation in the lowest phosphate concentration was explained by eGFR and PTH, further supporting the presence of one or more yet unidentified correlates. FGF23, not measured in this cohort, could at least partially further explain this variation.

The association between hypophosphatemia and lower cardiovascular mortality after transplantation can be reconciled with previous studies by our group and others. Early after successful kidney transplantation, when renal function is rapidly restored but FGF23 and PTH remain high, the resulting renal phosphate leak will lead to a subsequent decline in circulating FGF23 and PTH. This decline could be more efficient in those with hypophosphatemia than in those without hypophosphatemia. As FGF23 has been implicated in cardiovascular disease and associated with a higher cardiovascular mortality risk in renal transplant recipients, more efficient lowering of FGF23 may contribute to the lower risk of cardiovascular mortality in renal transplant recipients who developed hypophosphatemia in the first year after transplantation. The development of hypophosphatemia was associated with cardiovascular but not with non-cardiovascular mortality.

A limitation of our study is that we do not have data on FGF23 concentrations available, which precludes conclusions on the relationship between FGF23 and the severity of hypophosphatemia. PTH was only measured on indication, probably leading to an overrepresentation of patients with hyperparathyroidism in our data. However, adjusting for PTH in the available cases did not change the results. Another limitation is the unknown
cause of death in a relatively large proportion of patients. Cardiovascular disease was a cause of death in 32% of patients in our cohort, which is less common than previously observed\textsuperscript{30}. Probably cardiovascular mortality is overrepresented in the group of patients with an unknown cause of death. However, when we performed an exploratory analysis to find effects of the overrepresentation, the results were highly similar to the original analyses. Third, our patient population had a low incidence of diabetes mellitus and mainly consisted of Caucasians, which may limit the generalizability of our findings. On the other hand, strengths of our study include the design, taking advantage of a large number of intra-individual measurements, the high external validity due to the use of real-life data, and the complete and long-term follow-up.

In conclusion, we found that patients who develop hypophosphatemia after kidney transplantation are at lower risk of graft failure and cardiovascular mortality compared with patients who do not develop hypophosphatemia. Future studies should address whether patients who do not develop hypophosphatemia require more intense monitoring for accelerated renal function loss or cardiovascular disease.

**Acknowledgments**

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References


