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# Urinary Endotrophin and Long-term Outcomes in Kidney Transplant Recipients

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**Background.** Kidney fibrosis is a suggested cause of kidney failure and premature mortality. Because collagen type VI is closely linked to kidney fibrosis, we aimed to evaluate whether urinary endotrophin, a collagen type VI fragment, is associated with graft failure and mortality among kidney transplant recipients (KTR). **Methods.** In this prospective cohort study, KTR with a functioning graft ≥1-y posttransplantation were recruited; 24-h urinary endotrophin excretion was measured using an ELISA method. Multivariate Cox regression analyses were performed. **Results.** A total of 621 KTR (mean age 53 y old, 43% female) at a median of 5.2 y posttransplantation were included. Median 24-h urinary endotrophin excretion was 5.6 (3.1–13.6) µg/24h. During a median follow-up of 7.5 y, 87 KTR (14%) developed graft failure and 185 KTR (30%) died; 24-h urinary endotrophin excretion was associated with increased risk of graft failure (hazard ratio [95% confidence interva] per doubling = 1.24 [1.08-1.42]) and all-cause mortality (hazard ratio [95% confidence intervals] per doubling = 1.14 [1.03-1.25]) independent of potential confounders including plasma endotrophin concentration. Twenty-four-hour urinary protein excretion was a significant effect modifier for the association with mortality ($P_{interaction} = 0.002$). Twenty-four-hour urinary endotrophin excretion was only significantly associated with mortality in KTR with low levels of proteinuria. **Conclusions.** Urinary endotrophin is independently associated with an increased risk of graft failure in all KTR and mortality only in KTR with low levels of proteinuria. Further studies with different KTR populations are needed to confirm these findings.

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Kidney transplantation is the preferred treatment option for patients with end-stage kidney disease. In the past 60 y, short-term outcomes after kidney transplantation have greatly improved. Unfortunately, the long-term outcomes remain limited because of the risks of graft failure and premature mortality.[1,2] One important contributor that

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*Public sharing of individual participant data was not included in the informed consent of the TransplantLines Biobank and Cohort Study, but data can be made available to interested researchers upon reasonable request by sending an e-mail to the data manager of the TransplantLines Biobank and Cohort study (datarequest.transplantlines@umcg.nl).*

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hampers the long-term outcomes of kidney transplantation is fibrosis.[3]

Fibrosis reflects a pathological response to the cumulative burden of injuries that exceeds the potential for restoration. This process is characterized by an imbalance between extracellular matrix components (ECM) formation and degradation.[4,5] In the kidney transplantation setting, various factors can cause kidney allograft injury, such as alloimmune responses to the graft, ischemia/reperfusion injury, and calcineurin-induced nephrotoxicity.[6,7] Regardless of the initiating injury, fibrosis in the kidney can lead to loss of kidney function and ultimately to kidney failure.[8] Moreover, patients with active kidney fibrosis are also at higher risk of mortality.[9] Therefore, assessment of fibrosis progression by evaluating the active ECM formation may identify kidney transplant recipients (KTR) who are at higher risk of adverse long-term outcomes.

Collagen is one of the key components of the ECM. Under healthy conditions, collagen type VI (COL VI) is deposited in the kidney at relatively low levels.[10,11] High deposition markedly increased under fibrotic conditions,[11,12] including KTR with chronic forms of rejection.[3] When COL VI is produced and deposited into the ECM, the C5 domain of the α3-chain, that is, endotrophin, is cleaved off and released into circulation.[13] Because of this, it is reasonable to evaluate the possibility of using endotrophin as a biomarker for active COL VI formation.

Previously, urinary endotrophin has been shown to reflect the degree of kidney fibrosis in patients with IgA nephropathy and ANCA-associated vasculitis,[14,15] and it can also be used as a prognostic marker to identify patients with a higher risk of worse kidney function.[15,16] Furthermore, urinary endotrophin has also been shown to be associated with disease progression in patients with chronic kidney disease (CKD).[12] In the KTR population, a recent study reported that urinary endotrophin at 3 mo posttransplantation was associated with lower kidney function at 12 mo.[17] However, whether urinary endotrophin is associated with long-term outcomes in KTR has yet to be studied in detail. In this study, we aimed to analyze the association of urinary endotrophin with other clinical and biochemical parameters and adverse long-term outcomes, that is, graft failure and all-cause mortality.

## MATERIALS AND METHODS

This study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[18]

### Study Design and Population

We used data and samples from the TransplantLines Food and Nutrition Biobank and Cohort Study (NCT02811835). All adult KTR with a functioning graft ≥1 y who visited the University Medical Center Groningen outpatient clinic between November 2008 and March 2011 were invited to participate. In total, 707 KTR agreed to participate and gave written informed consent.[19] For this study, 86 KTR with missing 24-h urinary endotrophin excretion were excluded, leaving 621 KTR with data available on 24-h urinary endotrophin excretion.

The study endpoints were graft failure (ie, the need for retransplantation or reinitiation of dialysis) and all-cause mortality. For graft failure, KTR who died with a functioning graft were censored at the time of death. Endpoints were recorded until December 2017. No participants were lost to follow-up. The same study included an additional population of potential kidney donors before donation as a healthy control group. In total, 300 potential living kidney donors agreed to participate and gave written informed consent.[19] For this study, 135 potential living kidney donors with missing 24-h urinary endotrophin excretion were excluded, leaving 165 of them with data available on 24-h urinary endotrophin excretion.

This study was approved by the institutional review board of University Medical Center Groningen (METc 2008/186) and adhered to the Declarations of Helsinki and Istanbul.

### Kidney Transplant Characteristics and Data Collection

All KTR who underwent transplantation at the UMCG were treated with standard immunosuppressive therapy. Standard immunosuppression regiment consisted of the following: azathioprine (100 mg/d) and prednisolone (starting with 20 mg/d and tapering to 10 mg/d) from 1968 to 1989; cyclosporine (target trough levels 175–200 mg/L in the first 3 mo, 100 mg/L thereafter) and prednisolone (starting with 20 mg/d and tapering to 10 mg/d) from 1989 to 1996. In 1997, mycophenolate mofetil (2 g/d) was added to the standard immunosuppressive regimen. For KTR with no complications, cyclosporine was slowly withdrawn from 1-y posttransplantation onward. In 2012, cyclosporine was replaced by tacrolimus, and KTRs continued triple-immunosuppressive therapy with prednisolone (20 mg/d, tapering to 5 mg/d), tacrolimus (target trough levels 8–12 μg/L in the first 3 mo, 6–10 μg/L until month 6, and 4–6 μg/L from 6 mo onward), and mycophenolate mofetil (starting with 2 g/d, tapering to 1 g/d).[20]

Baseline clinical data were collected during a visit to the outpatient clinic, and relevant recipient, donor, and transplant information were extracted from the medical records.[19] The estimated glomerular filtration rate (eGFR) was calculated using the creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[21] Delayed graft function (DGF) was defined as the need for at least one dialysis treatment within the first week after kidney transplantation.[22]

### Laboratory Measurements

KTR were instructed to collect 24-h urine the day before they visited the outpatient clinic. On the day of the visit, fasting blood samples were withdrawn.[19] Upon collection, samples were aliquoted and kept frozen at −80°C until analysis. Urinary and plasma endotrophin were measured using an ELISA developed at Nordic Bioscience (Herlev, Denmark). This ELISA kit uses a monoclonal antibody that specifically detects the last 10 amino acids of the alpha-3 chain of COL VI, that is, released upon COL VI deposition in the extracellular matrix.[23] Other biochemical parameters were measured using routine spectrophotometric methods (Roche Diagnostics, Basel, Switzerland). The measurements of biochemical parameters, including urinary and plasma endotrophin, were performed at a single time point after transplantation, which was at the baseline evaluation at least 1-y posttransplant in all study participants.

### Statistical Analyses

Data distribution of continuous variables was assessed by Quantile-Quantile plots. For descriptive statistics, data were presented as mean ± SD for variables with normal distribution, median (interquartile range) for variables with skewed distribution, and frequency (valid percentage) for categorical variables. Differences in 24-h urinary endotrophin excretion between KTR and healthy donors were statistically tested using the Mann-Whitney *U* test. Differences at baseline between subgroups of KTR according to tertiles of 24-h urinary endotrophin excretion were tested by 1-way ANOVA for continuous variables with normal distribution, Kruskall-Wallis test for continuous variables with skewed distribution, and $\chi^2$ test for categorical variables.

Linear regression analyses were performed to assess the associations between 24-h endotrophin excretion and clinical and biochemical parameters. During the linear regression analyses, variables with skewed distribution were $\log_2$-transformed to fulfill the assumption for linear regression. Two variables, time after transplantation and urinary protein excretion, did not fulfill the assumption after $\log_2$-transformation. Therefore, we categorized the time after transplantation variable into 2 categories based on the median graft survival of the study population (≤12.5 y versus >12.5 y), and urinary protein excretion was categorized into 2 categories using the cutoff value that was recommended by the American Society of Transplantation for the outpatient surveillance of KTR (≤0.5 g/24h versus >0.5 g/24h).[24]

Graft and patient survival across tertiles of 24-h urinary endotrophin were visualized using Kaplan-Meier curves, and the significance of the differences between tertiles was assessed using log-rank tests. Cox proportional-hazard regression analyses were used to assess the association of 24-h urinary endotrophin excretion with graft failure and all-cause mortality. Several adjustments were performed to account for the effect of potential confounders. In model 1, we adjusted for age, sex, and time after transplantation. In model 2, we further adjusted for eGFR. In model 3, we further adjusted for $\log_2$ 24-h urinary protein excretion. In model 4, we further adjusted for body mass index (BMI) and diabetic nephropathy as the primary kidney disease. In model 5, we further adjusted for donor age and previous history of DGF. In model 6 (full model), we further adjusted for $\log_2$ plasma endotrophin. Schoenfeld residuals were tested, and the full models did not violate the assumption for proportionality of hazards ($P = 0.5$ for graft failure and $P = 0.7$ for all-cause mortality).

Potential effect modifications by age, sex, plasma endotrophin, eGFR, and 24-h urinary protein excretion were tested by fitting both main effects and their cross-product terms in the full model, where $p_{interaction} < 0.05$ indicated a potential effect modification. As we identified potential effect modification by 24-h urinary protein excretion for the association between 24-h urinary endotrophin excretion and all-cause mortality, we repeated the full model Cox regression analyses in subgroups based upon the cutoff value of 24-h urinary protein excretion that has been recommended by the American Society of Transplantation (0.5 g/24h).[24] Although this cutoff is most commonly used in clinical practice, other cutoff values for proteinuria have also been used in literature, that is, 0.15 g/24h, 1.0 g/24h, and 1.5 g/24h.[25,26] To investigate whether the finding of effect modification is consistent independent of the cutoff value applied, we also performed the subgroup analyses using these cutoffs.

For sensitivity analyses, we evaluated the association of 24-h urinary endotrophin excretion with study endpoints after excluding outliers. Outliers were defined as values deviating >2 SD from the mean of the $\log_2$ 24-h urinary endotrophin excretion.[27] Furthermore, since urinary endotrophin has been shown to reflect the degree of fibrosis in nontransplant patients with kidney disease,[14,15] we reevaluated the association of 24-h urinary endotrophin excretion with study endpoints after excluding KTR with recurrent kidney disease as the cause of graft failure. Next to that, we repeated the Cox regression analyses with urinary endotrophin concentration and urinary endotrophin concentration indexed for creatinine (urinary endotrophin/creatinine ratio). Additionally, since diabetes is a systemic disease and it is independently associated with mortality,[28,29] and because endotrophin has been shown to be associated with diabetes,[30,31] we repeated the Cox regression analyses in subgroups based on diabetes status for the association between 24-h urinary endotrophin excretion and mortality.

For all cross-sectional analyses, the original dataset was used, and variables with >20 missing values were reported in the table footnotes. For all prospective analyses, multiple imputations were performed to account for missing data other than 24-h urinary endotrophin excretion. All statistical analyses were performed using R version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria). A 2-sided $P < 0.05$ was considered significant for all analyses.

## RESULTS

The flow diagram of the study population is presented in **Figure S1** (**SDC**, http://links.lww.com/TXD/A623). In total, 621 KTR (age 53 ± 13 y old, 43% female) were included in the analyses. The 24-h urinary endotrophin excretion at baseline was 5.6 (3.1–13.6) µg/24h. This was significantly higher than the control group of 165 healthy controls (age 54 ± 11 y old, 55% female), in whom the 24-h urinary endotrophin excretion was 4.3 (3.1–5.5) µg/24h ($P < 0.001$; **Figure 1**); 24-h urinary endotrophin excretion significantly correlated with plasma endotrophin concentration in KTR but not in healthy controls. Nevertheless, plasma endotrophin only contributed to 20% of the variance in the 24-h urinary endotrophin excretion among KTR (**Figure S2, SDC**, http://links.lww.com/TXD/A623).

### Baseline Characteristics and Associations of 24-h Urinary Endotrophin Excretion With Clinical and Biochemical Parameters

The median time after transplantation was 5.2 (2.0–12.0) y, and the eGFR was 52 ± 20 mL/min/1.73 m$^2$. Stratified based on the tertiles of 24-h urinary endotrophin excretion, BMI, donor age, the prevalence of previous history of DGF, serum creatinine, and urinary protein excretion increased, whereas female prevalence and time after transplantation decreased across increasing tertiles. More detailed baseline characteristics of the KTR are presented in Table 1. Since there was a significant difference in sex across tertiles, we stratified the tertiles with an adjustment for sex. The differences across tertiles were similar after the sex stratification (**Table S1, SDC**, http://links.lww.com/TXD/A623).

In univariable linear regression analyses, BMI, hypertension, donor age, history of rejection, and calcineurin inhibitor use were significantly associated with 24-h urinary

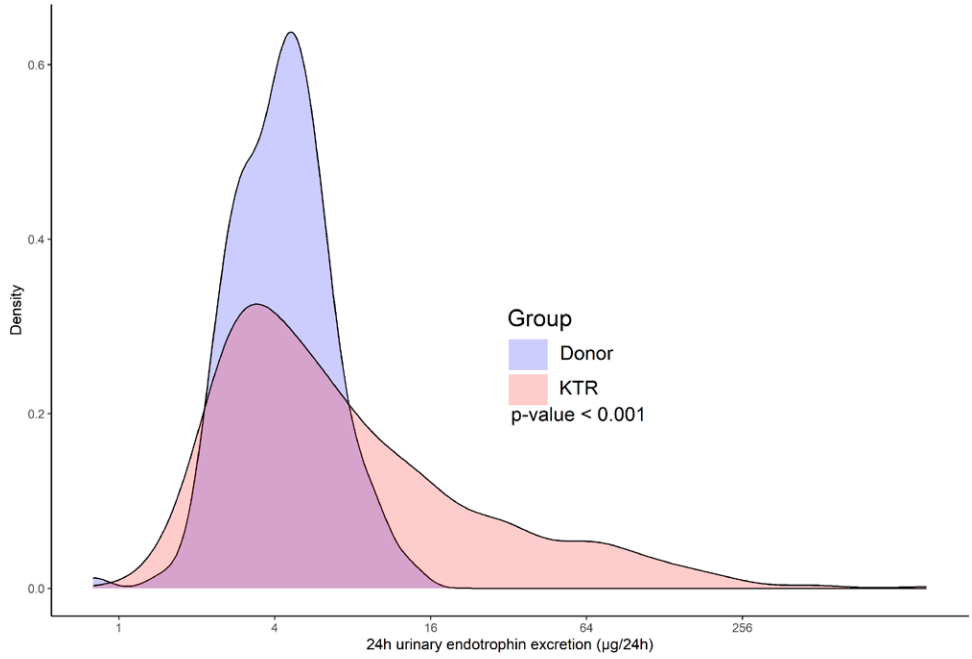


**FIGURE 1.** Density plot of 24-h urinary endotrophin excretion in donor and KTR. The *P* value between the donor and KTR was calculated using a Mann-Whitney *U* test. KTR, kidney transplant recipients.

endotrophin excretion; however, these associations were lost after adjustments with sex, serum creatinine, and 24-h urinary protein excretion. In multivariable regression analyses, the strongest association of 24-h urinary endotrophin excretion was with the previous history of DGF (Standardized $\beta = 0.45$, $P < 0.001$; Table 2).

## Prospective Associations of 24-h Urinary Endotrophin Excretion With Graft Failure

During a follow-up of 7.5 (5.1–8.2) y, 87 (14%) KTR developed graft failure. The most frequent cause of graft failure was rejection (77%). Other causes of graft failure included recurrence of primary kidney disease, vascular problems, and infection. Graft failure occurred in 14 (6.8%), 10 (4.8%), and 63 (30%) KTR in the first, second, and third tertile of 24-h urinary endotrophin excretion, respectively ($p_{log\text{-}rank} < 0.001$; Figure 2).

Cox regression analyses showed that being in the third tertile of 24-h urinary endotrophin excretion was associated with a higher risk of developing graft failure than being in the first or second tertile. The association remained significant albeit weakened after adjustment for potential confounders, including plasma endotrophin (hazard ratio [HR] [95% confidence interval (CI)] = 3.20 [1.88-5.44]). The results were consistent when 24-h urinary endotrophin excretion was analyzed as a continuous variable, with doubling of 24-h urinary endotrophin excretion being associated with a higher risk of graft failure (HR [95%CI] = 1.24 [1.08-1.42]) (Table 3). The full Cox model for the association of 24-h urinary endotrophin excretion with graft failure is presented in **Table S2** (**SDC**, http://links.lww.com/TXD/A623).

## Prospective Associations of 24-h Urinary Endotrophin Excretion With All-cause Mortality

During a follow-up of 7.5 (6.2–8.3) y, 185 (30%) KTR died. Death occurred in 50 (24%), 43 (21%), and 92 (44%) KTR in the first, second, and third tertile of 24-h urinary endotrophin excretion, respectively ($p_{log\text{-}rank} < 0.001$) (Figure 3). Cox regression analyses showed that being in the third tertile of 24-h urinary endotrophin excretion was associated with a higher risk of all-cause mortality than being in the first or second tertile, and the association remained significant after adjustment for potential confounders including plasma endotrophin (HR [95% CI] = 1.43 [1.15-1.77]). The results were consistent when 24-h urinary endotrophin excretion was analyzed as a continuous variable, with doubling of 24-h urinary endotrophin excretion being associated with all-cause mortality (HR [95% CI] = 1.14 [1.03-1.25]) (Table 4). Furthermore, the association with all-cause mortality was not driven by graft failure (**Table S3, SDC**, http://links.lww.com/TXD/A623).

As we identified an interaction between 24-h urinary endotrophin excretion and 24-h urinary protein excretion for the association with all-cause mortality ($p_{interaction} = 0.002$), we performed subgroup analyses in the full model based on the 24-h urinary protein excretion level; 24-h urinary endotrophin excretion was only associated with all-cause mortality in KTR with low levels of proteinuria, regardless of whether 0.5 g/24h or other cutoff values were used (Figure 4). The full Cox model for the association of 24-h urinary endotrophin excretion with all-cause mortality in the subgroup analyses stratified based on the 24-h urinary protein excretion cutoff value that was recommended by the American Society of Transplantation (0.5 g/24h) is presented in **Table S4** (**SDC**, http://links.lww.com/TXD/A623).

## Sensitivity Analyses

There were 34 (5.5%) KTR with 24-h urinary endotrophin excretion above the 2SD from the mean of the $\log_2$ 24-h urinary endotrophin excretion, and no KTR with 24-h urinary endotrophin below the 2SD. After excluding these outliers, the association of 24-h urinary endotrophin excretion with graft failure remained unchanged (**Table S5, SDC**,

**TABLE 1.**

**Baseline characteristics**

| Variables | Total N = 621 | Tertile 1 N = 207 <3.8 µg/24h | Tertile 2 N = 207 3.8–9.44 µg/24h | Tertile 3 N = 207 >9.44 µg/24h | *P* |
|---|---|---|---|---|---|
| 24-h urinary endotrophin excretion, µg/24h | 5.6 (3.1–13.6) | 2.8 (2.4–3.1) | 5.6 (4.5–6.8) | 23 (14–56) | |
| **Clinical characteristics** | | | | | |
| Female sex, n (%) | 266 (43) | 99 (48) | 98 (47) | 69 (33) | **0.003** |
| Age, y | 53 ± 13 | 54 ± 13 | 53 ± 13 | 52 ± 13 | 0.4 |
| Primary kidney disease, n (%) | | | | | **0.011** |
| Hypertension | 28 (4.5) | 8 (3.9) | 5 (2.4) | 15 (7.2) | |
| Glomerulonephritis | 165 (26.6) | 65 (31.4) | 48 (23.2) | 52 (25.1) | |
| Interstitial nephritis | 77 (12.4) | 25 (12.1) | 25 (12.1) | 27 (13.0) | |
| Cystic kidney disease | 128 (20.6) | 31 (15.0) | 50 (24.2) | 47 (22.7) | |
| Other congenital/hereditary disease | 34 (5.5) | 14 (6.8) | 9 (4.3) | 11 (5.3) | |
| Diabetic nephropathy | 28 (4.5) | 8 (3.9) | 5 (2.4) | 15 (7.2) | |
| Other multisystem diseases | 45 (7.2) | 15 (7.2) | 19 (9.2) | 11 (5.3) | |
| Other | 19 (3.1) | 11 (5.3) | 5 (2.4) | 3 (1.4) | |
| Unknown | 97 (15.6) | 30 (14.5) | 41 (19.8) | 26 (12.6) | |
| BMI, kg/m$^2$ | 26.5 ± 4.7 | 25.7 ± 4.4 | 26.9 ± 4.9 | 27.0 ± 4.7 | **0.007** |
| SBP, mm Hg | 136 ± 17 | 134 ± 18 | 135 ± 16 | 137 ± 17 | 0.2 |
| Diabetes, n (%) | 140 (23) | 44 (21) | 37 (18) | 59 (29) | **0.030** |
| Hypertension, n (%) | 252 (41) | 79 (38) | 80 (39) | 93 (45) | 0.3 |
| History of cardiovascular disease, n (%) | 150 (24) | 58 (28) | 40 (19) | 52 (25) | 0.1 |
| Current smoking, n (%) | 68 (12) | 20 (10) | 20 (10) | 28 (15) | 0.3 |
| **Transplant-related characteristics** | | | | | |
| First kidney transplant, n (%) | 557 (90) | 184 (89) | 189 (92) | 184 (89) | 0.5 |
| Preemptive transplant, n (%) | 96 (16) | 39 (19) | 34 (16) | 23 (11) | 0.084 |
| Time after transplantation, y | 5.2 (2.0–12.0) | 7.5 (3.7–14.8) | 5 (2.2–10.6) | 4.5 (1.4–10.2) | **<0.001** |
| Time after transplantation >12. 5 y, n (%) | 142 (23) | 64 (31) | 41 (20) | 37 (18) | **0.003** |
| Donor age, y | 43 ± 16 | 40 ± 15 | 43 ± 15 | 46 ± 16 | **<0.001** |
| Living donor, n (%) | 211 (34) | 66 (32) | 71 (34) | 74 (36) | 0.7 |
| Positive HLA class I antibodies, n (%) | 99 (16) | 25 (12) | 33 (16) | 41 (20) | 0.1 |
| Positive HLA class II antibodies, n (%) | 109 (18) | 33 (16) | 35 (17) | 41 (20) | 0.6 |
| History of delayed graft function, n (%) | 47 (7.6) | 8 (3.9) | 10 (4.8) | 29 (14) | **<0.001** |
| History of rejection, n (%) | 165 (27) | 49 (24) | 49 (24) | 67 (32) | 0.069 |
| **Immunosuppressive medication** | | | | | |
| Prednisolone, n (%) | 614 (99) | 205 (99) | 205 (99) | 204 (99) | 0.9 |
| Calcineurin inhibitor, n (%) | 359 (58) | 101 (49) | 121 (59) | 137 (66) | **0.002** |
| Proliferation inhibitor, n (%) | 515 (83) | 175 (85) | 166 (80) | 174 (84) | 0.4 |
| mTOR inhibitor, n (%) | 24 (3.9) | 7 (3.4) | 9 (4.3) | 8 (3.9) | 0.9 |
| **Laboratory measurements** | | | | | |
| HbA1c, % | 5.8 (5.5–6.2) | 5.8 (5.5–6.1) | 5.8 (5.5–6.1) | 5.8 (5.5–6.2) | 0.9 |
| HbA1C > 6.5%, n (%) | 83 (13) | 27 (13) | 25 (12) | 31 (15) | 0.7 |
| hs-CRP, mg/L | 1.60 (0.70–4.40) | 1.40 (0.65–3.15) | 1.50 (0.70–3.85) | 1.90 (0.80–5.65) | **0.019** |
| Plasma endotrophin, ng/mL | 11.7 (9.2–15.5) | 10.1 (8.5–12.5) | 11.1 (8.8–14.3) | 15 (11–19) | **<0.001** |
| Serum creatinine, µmol/L | 125 (101–160) | 108 (90–126) | 118 (99–146) | 164 (135–200) | **<0.001** |
| eGFR, mL/min/1.73 m$^2$ | 52 ± 20 | 62 ± 18 | 55 ± 19 | 41 ± 17 | **<0.001** |
| Urinary albumin excretion, mg/24h | 42 (11–194) | 21 (6–106) | 26 (10–81) | 110 (31–400) | **<0.001** |
| Urinary protein excretion, g/24h | 0.21 (0.01–0.40) | 0.13 (0.01–0.29) | 0.17 (0.01–0.28) | 0.30 (0.20–0.71) | **<0.001** |
| Urinary protein excretion >0.5 g/24h, n (%) | 142 (23) | 31 (15) | 30 (15) | 81 (39) | **<0.001** |

Smoking status was missing in 42 (6.8%) patients. Normally distributed variable was presented as mean ± SD, skewed variable was presented as median (interquartile range), and categorical variable was presented as nominal (valid percentage).
Significant values ($P < 0.05$) are in bold.
BMI, body mass index; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate based on creatinine-based CKD-EPI formula; hs-CRP, high-sensitivity C-reactive protein; mTOR, mechanistic target of rapamycin; SBP, systolic blood pressure.

http://links.lww.com/TXD/A623). Next, there were 6 KTR with recurrent kidney disease as the cause of graft failure. After excluding these KTR from analyses, the association of 24-h urinary endotrophin excretion with graft failure remained materially unchanged (**Table S6, SDC**, http://links.lww.com/TXD/A623). Additionally, we used urinary endotrophin/creatinine ratio and urinary endotrophin concentration instead of 24-h urinary endotrophin excretion. Both urinary endotrophin/creatinine ratio and urinary endotrophin concentration were independently associated with graft failure, either when presented as a continuous variable or as tertiles (**Tables S7 and S8**, http://links.lww.com/TXD/A623).

**TABLE 2.**

**Linear regression analyses of $log_2$ 24-h urinary endotrophin excretion**

| | Unadjusted linear regression | | Adjusted for sex, serum creatinine, and urinary protein excretion > 0.5 g/24h | |
|---|---|---|---|---|
| **Variables** | **St. β (95% CI)** | ***P*** | **St. β (95% CI)** | ***P*** |
| **Clinical characteristics** | | | | |
| Female sex | −0.23 (−0.38 to −0.07) | **0.005** | – | – |
| Age, y | −0.05 (−0. 13 to 0.03) | 0.2 | 0.02 (−0. 04 to 0.09) | 0.5 |
| Primary kidney disease | | | | |
| Hypertension | Ref | Ref | Ref | Ref |
| Glomerulonephritis | −0.44 (0. 84 to 0.04) | **0.030** | −0.29 (−0. 61 to 0.03) | 0.078 |
| Interstitial nephritis | −0.40 (−0. 83 to 0.03) | 0.068 | −0.14 (−0. 48 to 0.21) | 0.4 |
| Cystic kidney disease | −0.20 (−0. 60 to 0.21) | 0.3 | −0.05 (−0. 38 to 0.28) | 0.8 |
| Other congenital/hereditary disease | −0.43 (−0. 93 to 0.07) | 0.089 | −0.24 (−0. 64 to 0.16) | 0.2 |
| Diabetic nephropathy | 0.25 (−0. 27 to 0.77) | 0.3 | 0.24 (−0. 18 to 0.67) | 0.3 |
| Other multisystem diseases | −0.50 (−0.97 to −0.04) | **0.035** | −0.26 (−0. 64 to 0.12) | 0.2 |
| Other | −0.67 (−1.25 to −0.10) | **0.023** | −0.41 (−0. 87 to 0.06) | 0.089 |
| Unknown | −0.43 (−0.84 to −0.01) | **0.044** | −0.14 (−0. 48 to 0.20) | 0.4 |
| BMI, kg/m$^2$ | **0.10 (0. 02–0.18)** | **0.015** | 0.05 (−0. 01 to 0.11) | 0.1 |
| SBP, mm Hg | 0.12 (0. 04–0.19) | **0.004** | 0.04 (−0.02 to 0.11) | 0.2 |
| Diabetes | 0.14 (−0. 05–0.33) | 0.1 | **0.16 (0. 01 to 0.32)** | **0.036** |
| Hypertension | 0.17 (0. 01 to 0.33) | **0.033** | 0.06 (−0. 08–0.19) | 0.4 |
| History of cardiovascular disease | −0.04 (−0. 22–0.15) | 0.7 | −0.05 (−0. 20–0.10) | 0.5 |
| Current smoking | 0.18 (−0. 07–0.44) | 0.2 | 0.05 (−0. 16–0.25) | 0.7 |
| **Transplant-related** | | | | |
| First kidney transplant | 0.05 (−0. 21–0.31) | 0.7 | 0.04 (−0. 17–0.25) | 0.7 |
| Preemptive transplant | −0.19 (−0. 41–0.03) | 0.085 | −0.16 (−0. 33–0.02) | 0.078 |
| Time after transplantation > 12. 5 y | **−0.23 (−0.42 to −0.05)** | **0.015** | **−0.28 (−0.43 to −0.13)** | **<0.001** |
| Donor age, y | **0.15 (0. 07 to 0.23)** | **<0.001** | 0.04 (−0. 02–0.11) | 0.2 |
| Living donor | 0.03 (−0. 13–0.20) | 0.7 | 0.07 (−0. 06–0.21) | 0.3 |
| Positive HLA class I antibodies | **0.24 (0. 02 to 0.45)** | **0.031** | **0.18 (0. 01 to 0.36)** | **0.041** |
| Positive HLA class II antibodies | 0.19 (−0. 02–0.40) | 0.074 | 0.00 (−0. 17–0.17) | 1.0 |
| History of delayed graft function | **0.66 (0. 37 to 0.96)** | **<0.001** | **0.45 (0. 21 to 0.69)** | **<0.001** |
| History of rejection | **0.20 (0. 02 to 0.37)** | **0.031** | −0.01 (−0. 15–0.14) | 0.9 |
| **Immunosuppressive medication** | | | | |
| Prednisolone | −0.26 (−1. 00–0.49) | 0.5 | −0.16 (−0. 76–0.44) | 0.6 |
| Calcineurin inhibitor | **0.27 (0. 12 to 0.43)** | **0.001** | 0.04 (−0. 09–0.18) | 0.6 |
| Proliferation inhibitor | −0.05 (−0. 26–0.16) | 0.6 | 0.02 (−0. 15–0.19) | 0.8 |
| mTOR inhibitor | 0.11 (−0. 30–0.52) | 0.6 | 0.17 (−0. 16–0.50) | 0.3 |
| **Laboratory measurements** | | | | |
| HbA1c > 6.5% | 0.04 (−0. 19–0.27) | 0.7 | 0.13 (−0. 06–0.32) | 0.2 |
| hs-CRP, mg/L[a] | **0.13 (0. 05 to 0.21)** | **0.001** | **0.07 (0. 00 to 0.13)** | **0.044** |
| Plasma endotrophin, ng/mL[a] | **0.45 (0. 38 to 0.52)** | **<0.001** | **0.12 (0. 03 to 0.20)** | **0.008** |
| Serum creatinine, μmol/L[a] | **0.56 (0. 50 to 0.63)** | **<0.001** | – | – |
| eGFR, mL/min/1.73m$^2$ | **−0.48 (−0.55 to −0.41)** | **<0.001** | – | – |
| Urinary albumin excretion, mg/24h[a] | **0.32 (0. 25 to 0.40)** | **<0.001** | 0.01 (−0. 09–0.10) | 0.9 |
| Urinary protein excretion > 0.5g/24h | **0.79 (0. 62 to 0.97)** | **<0.001** | – | – |

[a]Variables were $log_2$-transformed to fulfill the assumption in linear regression.
Significant values ($P < 0.05$) are in bold.
BMI, body mass index; eGFR, estimated glomerular filtration rate based on creatinine-based CKD-EPI formula; HbA1c, hemoglobin A1c; hs-CRP, high-sensitivity C-reactive protein; HLA, human leukocyte antigen; mTOR, mechanistic target of rapamycin; SBP, systolic blood pressure.

We performed the same sensitivity analyses for the outcome of all-cause mortality. The association of 24-h urinary endotrophin excretion with all-cause mortality remained materially unchanged after the exclusion of outliers (**Table S9, SDC**, http://links.lww.com/TXD/A623) and after the exclusion of KTR with recurrent kidney disease (**Table S10, SDC**, http://links.lww.com/TXD/A623). Furthermore, both urinary endotrophin/creatinine ratio and urinary endotrophin concentration were also independently associated with all-cause mortality, either when presented as a continuous variable or as tertiles (**Tables S11 and 12, SDC**, http://links.lww.com/TXD/A623). Finally, we performed additional subgroup analyses for the outcome of all-cause mortality based on the diabetes status. The association of 24-h urinary endotrophin excretion with all-cause mortality was comparable between KTR with and without diabetes (**Figure S3, SDC**, http://links.lww.com/TXD/A623).

## DISCUSSION

In a large cohort of KTR, 24-h urinary endotrophin excretion was significantly higher compared with the healthy

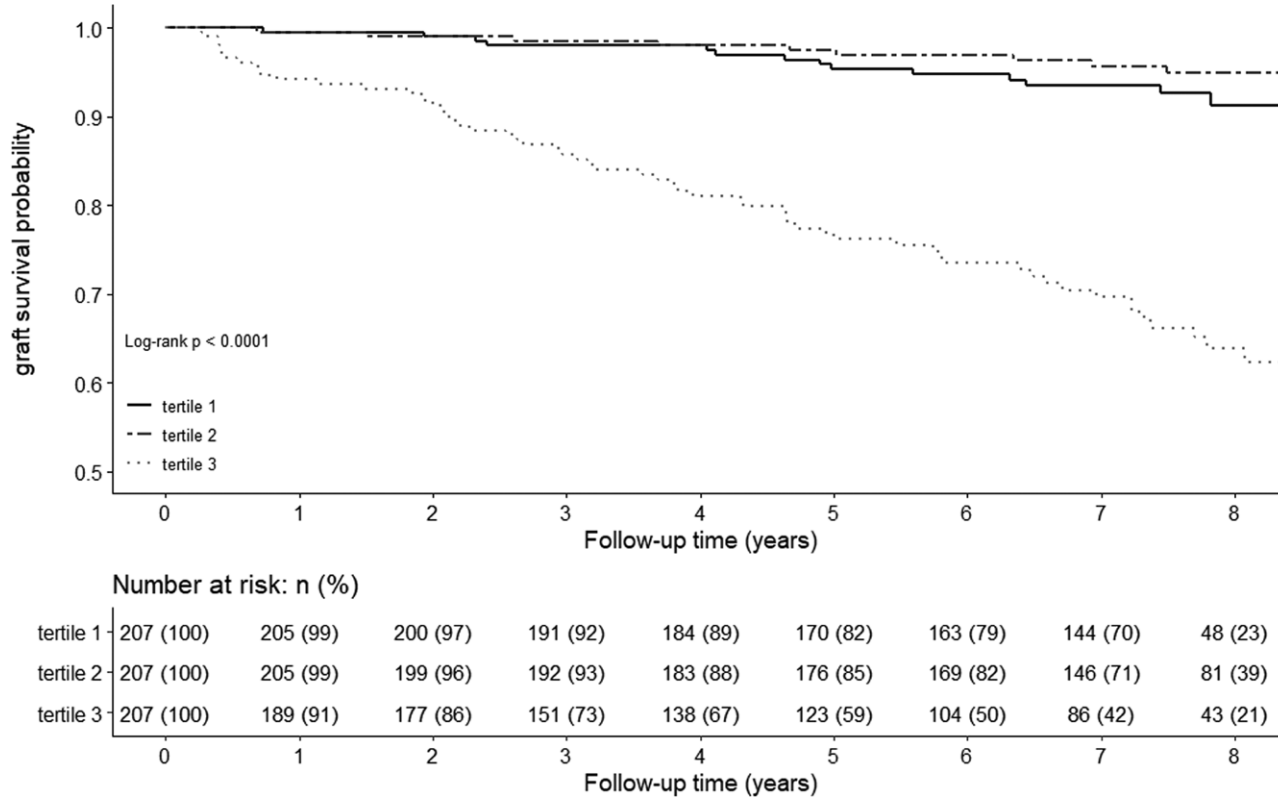


**FIGURE 2.** Kaplan-Meier analysis for death-censored graft survival per tertile of 24-h urinary endotrophin excretion.

**TABLE 3.**

**Prospective analyses of the association of 24-h urinary endotrophin excretion with death-censored graft failure in 621 kidney transplant recipients**

| | 24-h urinary endotrophin excretion | | | | |
|---|---|---|---|---|---|
| | Lowest 2 tertiles <9.45 µg/24h | Highest tertile ≥9.45 µg/24h | | Continuous (per doubling) | |
| $n_{events}$ | 24 | 63 | | 87 | |
| **Model** | | **HR (95% CI)** | ***P*** | **HR (95% CI)** | ***P*** |
| Crude | Ref | 6.87 (4.29-11.0) | <0.001 | 1.59 (1.45-1.74) | <0.001 |
| Model 1 | Ref | 7.40 (4.59-11.94) | <0.001 | 1.63 (1.48-1.78) | <0.001 |
| Model 2 | Ref | 3.67 (2.17-6.21) | <0.001 | 1.35 (1.20-1.52) | <0.001 |
| Model 3 | Ref | 2.98 (1.75-5.06) | <0.001 | 1.20 (1.07-1.36) | 0.003 |
| Model 4 | Ref | 2.98 (1.75-5.06) | <0.001 | 1.21 (1.06-1.37) | 0.004 |
| Model 5 | Ref | 3.23 (1.90-5.50) | <0.001 | 1.25 (1.09-1.43) | 0.001 |
| Model 6 | Ref | 3.20 (1.88-5.44) | <0.001 | 1.24 (1.08-1.42) | 0.002 |

In total, 87 (14%) kidney transplant recipients developed death-censored graft failure (the need for retransplantation or [re]initiation of dialysis) during a median follow-up time of 7.5 (5.1–8.2) y. Cox proportional-hazard regression analyses were performed to assess the association of 24-h urinary endotrophin excretion with the risk of death-censored graft failure. Model 1 was adjusted for age, sex, and time after transplantation at inclusion. Model 2 was further adjusted for estimated glomerular filtration rate based on the creatinine-based CKD-EPI formula. Model 3 was further adjusted for $\log_2$ 24-h urinary protein excretion. Model 4 was further adjusted for body mass index and diabetic nephropathy as primary kidney disease. Model 5 was further adjusted for donor age and history of delayed graft function. Model 6 was further adjusted for $\log_2$ plasma endotrophin.
95% CI, 95% confidence interval; HR, hazard ratio.

controls; 24-h urinary endotrophin excretion was associated with worse kidney function and more proteinuria. Furthermore, a previous history of DGF was strongly associated with 24-h urinary endotrophin excretion. Prospectively, 24-h urinary endotrophin excretion was associated with a higher risk of graft failure and all-cause mortality, independent of potential confounders including plasma endotrophin. The association with all-cause mortality was observed only in KTR with low levels of proteinuria.

Accumulating evidence has shown that endotrophin, measured in plasma or urine, is associated with CKD severity and prospectively with kidney function deterioration.[12,14,32] In the setting of kidney transplantation, plasma endotrophin is associated with lower kidney function and a higher risk of graft failure.[3,33] However, as previously mentioned by Scherer and Gupta (2021), it is difficult to evaluate whether the association of endotrophin in the circulation with kidney disease is caused by impaired kidney function or by an

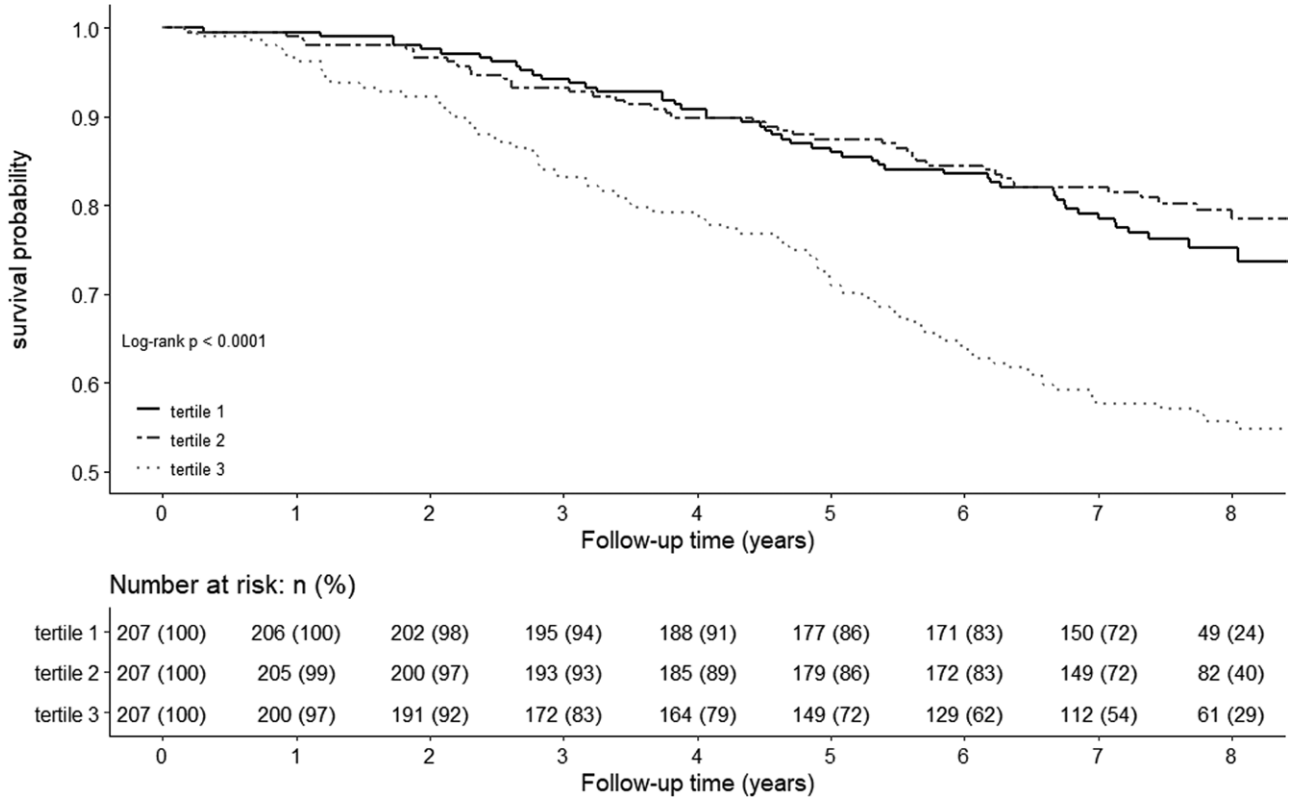


**FIGURE 3.** Kaplan-Meier analysis for patient survival per tertile of 24-h urinary endotrophin excretion.

**TABLE 4.**

**Prospective analyses of the association of 24-h urinary endotrophin excretion with all-cause mortality in 621 kidney transplant recipients**

| | 24-h urinary endotrophin excretion | | | | |
|---|---|---|---|---|---|
| | Lowest 2 tertiles <9.45 µg/24h | Highest tertile ≥9.45 µg/24h | | Continuous (per doubling) | |
| $n_{events}$ | 93 | 92 | | 185 | |
| **Model** | | **HR (95% CI)** | ***P*** | **HR (95% CI)** | ***P*** |
| Crude | Ref | 2.29 (1.72-3.06) | <0.001 | 1.22 (1.13-1.31) | <0.001 |
| Model 1 | Ref | 2.60 (1.93-3.49) | <0.001 | 1.27 (1.18-1.37) | <0.001 |
| Model 2 | Ref | 2.36 (1.70-3.26) | <0.001 | 1.24 (1.13-1.35) | <0.001 |
| Model 3 | Ref | 2.13 (1.52-2.98) | <0.001 | 1.18 (1.08-1.30) | <0.001 |
| Model 4 | Ref | 2.08 (1.48-2.92) | <0.001 | 1.16 (1.05-1.27) | 0.003 |
| Model 5 | Ref | 2.10 (1.49-2.96) | <0.001 | 1.15 (1.05-1.27) | 0.004 |
| Model 6 | Ref | 1.43 (1.15-1.77) | 0.001 | 1.14 (1.03-1.25) | 0.010 |

In total, 185 (30%) kidney transplant recipients died during a median follow-up time of 7.5 (6.2–8.3) y. Cox proportional-hazard regression analyses were performed to assess the association of 24-h urinary endotrophin excretion with risk of all-cause mortality. Model 1 was adjusted for age, sex, and time after transplantation at inclusion. Model 2 was further adjusted for estimated glomerular filtration rate based on the creatinine-based CKD-EPI formula. Model 3 was further adjusted for $\log_2$ 24-h urinary protein excretion. Model 4 was further adjusted for body mass index and diabetic nephropathy as primary kidney disease. Model 5 was further adjusted for donor age and history of delayed graft function. Model 6 was further adjusted for $\log_2$ plasma endotrophin.
95% CI, 95% confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; HR, hazard ratio.

actual increase in kidney fibrosis,[34] considering that endotrophin has a low molecular weight (~10–15 kDa),[35] and all low molecular weight protein is freely filtered in healthy individuals.[36]

In this study, we found that urinary endotrophin excretion was significantly correlated with its plasma concentrations. This finding is similar to the recently published study in the KTR population.[17] Nevertheless, plasma endotrophin can only explain 20% of the variance in the 24-h urinary endotrophin excretion among KTR. This suggests that the endotrophin measured in the urine originated from 2 sources, that is, systemic circulation and local production from the kidney, and the majority originates from the latter. In line with this, we found that the association of 24-h urinary endotrophin excretion with graft failure remained significant even after further adjustment with plasma endotrophin, indicating that endotrophin excretion is partially related to intrarenal fibrotic processes.

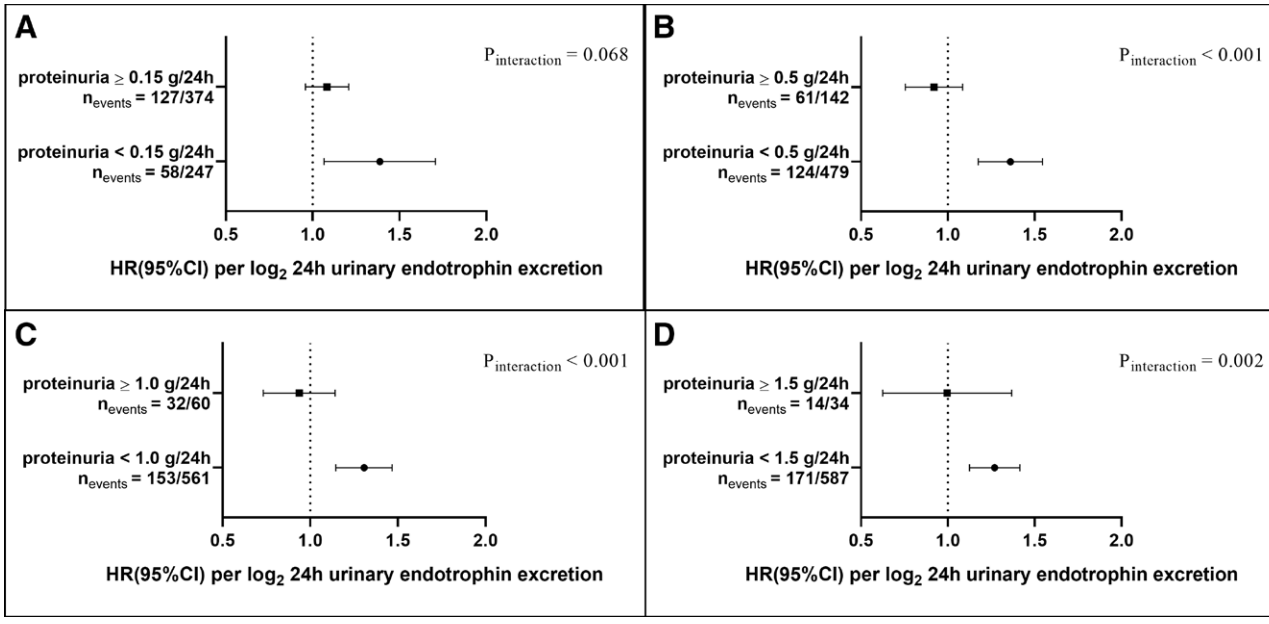


**FIGURE 4.** Forest plot for the association of 24-h urinary endotrophin excretion with all-cause mortality in subgroups based on the 24-h urinary protein excretion level: (A) 0.15 g/24h; (B) 0.5 g/24h; (C) 1.0 g/24h; (D) 1.5 g/24h. The model was adjusted for the full model of age, sex, time after transplantation at inclusion, estimated glomerular filtration rate based on creatinine-based CKD-EPI formula, $\log_2$ 24-h urinary protein excretion, body mass index, diabetic nephropathy as the primary kidney disease, donor age, history of delayed graft function, and $\log_2$ plasma endotrophin. CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

Urinary endotrophin was also independently associated with mortality, and this association was not driven by graft failure. Furthermore, the HR of urinary endotrophin excretion with mortality was similar in KTR with and without diabetes. Even before graft failure, the mortality risk in patients with impaired kidney function has increased.[37,38] Our finding was in contrast to findings from previous studies, where the association with mortality was only observed with plasma but not urinary endotrophin.[9,39,40] Differences in the study populations can potentially explain the discrepancy between our study and previous studies. All previous studies included nontransplant patients with type 2 diabetes as the study population, whereas this study included KTR. Although mortality risk in patients with type 2 diabetes is known to be 2 to 3 times higher than subjects in the general population, this risk is known to be approximately 6 to 7 times higher in KTR, with this elevated risk being independent of the level of kidney function.[41,42]

The association between 24-h urinary endotrophin excretion and all-cause mortality was observed solely among KTR with low levels of proteinuria, regardless of the cutoff values used to define proteinuria. Indeed, proteinuria has been noted as an important risk factor for mortality in KTR.[26] However, there are other risk factors for mortality in the KTR population, including recipient age and sex, diabetic nephropathy as the primary kidney disease, recipient BMI, and donor age.[43] Therefore, there is a need for biomarkers to identify KTR who are at higher risk of mortality, even in KTR with low levels of proteinuria. For this, urinary endotrophin measurement might be a promising candidate. By employing biomarkers such as urinary endotrophin, clinicians may identify patients who require more aggressive monitoring and targeted therapeutic interventions earlier so that disease progression can be prevented, survival rates can be improved, and the healthcare burdens can be reduced.

Another reason why it is of interest to assess endotrophin levels is that endotrophin is not just an end product of COL VI but is also a biologically active peptide. Endotrophin is able to promote inflammation and fibrosis by recruiting macrophages, stimulating transforming growth factor β expression, and promoting epithelial-mesenchymal transition.[30,35,44] Reducing levels of endotrophin may therefore be favorable. Previous studies have shown that different treatment modalities reduce levels of endotrophin, such as glucagon-like peptide-1 receptor agonists[45] or a tyrosine kinase inhibitor.[46]

There are several limitations in this study. This study was performed in a single center in the Netherlands with an overrepresentation of the Caucasian population; therefore, the findings in this study need to be externally validated in other KTR populations with different ethnicities. Another possible limitation of our study is that 24-h urinary endotrophin excretion was assessed at a single time point at baseline and that we did not use repeated measurements of 24-h urinary endotrophin excretion in our analyses, by which we could have accounted for potential waxing and waning of the endotrophin excretion of time. However, most epidemiologic studies like the current one use single baseline measurements to investigate associations with long-term outcomes. The use of a single value instead of repeated measurements adversely affects the strength of associations because taking into account intraindividual variability of parameters results in stronger rather than weaker associations with long-term outcomes.[47,48] Therefore, associations for 24-h urinary endotrophin excretion with outcome would probably have been stronger if we could have used repeated measurements in our analyses, by which we could have accounted for potential waxing and waning of the endotrophin excretion over time. However, this was not possible because measurement of variables, including 24-h urinary endotrophin excretion, was only

performed at baseline and not repeatedly. Serial endotrophin monitoring may be a direction for future studies. Next to that, the 24-h urinary endotrophin excretion measurements are made at different times after kidney transplantation. Although this may raise concern and may be seen as a study limitation, the justification for this is that we wanted to evaluate the association of the biomarker with long-term outcomes in the real-world outpatient clinical setting, in which patients do not visit the outpatient clinic only at a specific point in time, for example, at 1-y posttransplantation, but at different times after kidney transplantation. Furthermore, as this was an observational study, the nature of this study did not allow us to infer causality. Finally, residual confounding may still exist despite the number of potentially confounding factors we had adjusted for.

In conclusion, this study shows that 24-h urinary endotrophin excretion is elevated among KTR and is associated with an increased risk of graft failure in all KTR and all-cause mortality in KTR with low levels of proteinuria, independent of potential confounders including plasma endotrophin. These findings suggest a potential role of urinary endotrophin as a biomarker for long-term outcomes evaluation among KTR.

## ACKNOWLEDGMENTS

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