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A Lower Ratio of Uromodulin to Osteopontin in Deceased Donor Urine is Associated with Favorable Kidney Graft Outcomes

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Abstract

Deceased-donor kidneys experience extensive injury, activating (mal)adaptive pathways. Uromodulin (UMOD) and osteopontin (OPN) are produced in the kidney after ischemia, and may play a role in (mal)adaptive pathways and graft outcomes.

We measured urine UMOD and OPN in 1298 deceased-donors at organ procurement and determined the association of UMOD to OPN ratio with death-censored graft failure (dcGF) in 2430 recipients. Given that our ratio was data-driven, we internally validated the associations in training and test datasets. Furthermore, to understand the mechanism behind the identified associations, we examined the effect of UMOD on the expression of major histocompatibility complex II (MHCII) in mouse macrophages.

During a median (IQR) follow-up of 4 (3, 5) years, 13% experienced dcGF [33 (30–37) per 1000 patient-years]. Doubling of UMOD minimally increased risk for dcGF with an adjusted hazard ratio (aHR) of 1.1 (1.02–1.2), whereas doubling of OPN decreased the risk of dcGF [aHR 0.94 (0.88–1)]. UMOD-to-OPN ratio ≤ 3 improved the strength of association with 43% decreased risk of dcGF [aHR 0.57 (0.41–0.80)] and similar findings in the test dataset. In the preclinical model UMOD increased MHCII expression on macrophages elucidating a possible mechanism behind UMOD's association with increased graft failure.

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The UMOD-to-OPN ratio may help characterize the (mal)adaptive processes in deceased-donor kidneys and a lower ratio was associated with lower graft failure.

Keywords

uromodulin; tamm-horsfall protein; osteopontin; deceased donor; kidney; graft failure; adaptive; maladaptive

Introduction

Deceased donors undergo extensive biological changes with simultaneous activation of both injury and recovery processes in response to ischemia.[1] Neurogenic hypotension and systemic up-regulation of pro-inflammatory markers follow brain death (the predominant process by which death occurs in deceased donors in the US), resulting in direct renal injury secondary to ischemia and reperfusion as well as up-regulation of inflammatory pathways.[2–4] Along with injury and inflammation, there is also initiation of both adaptive and maladaptive processes in the kidney.[5, 6] We hypothesize that while these adaptive and maladaptive mechanisms are initiated in donor kidneys prior to implantation, they have a durable impact on subsequent graft function with either resolution of parenchymal injury and favorable long-term sequelae or accelerated fibrosis and reduction in graft function, respectively. Interrogating these pathways may aid in predicting recipient graft function, which would help further risk stratify deceased-donor kidneys for appropriate allocation. Markers such as YKL-40 have been shown to have a protective effect in the deceased–donor kidney transplant setting, correlating with reduced graft failure and improved 6-month graft function.[6] These findings indicate the need to investigate other related biological pathways that may affect the trajectory of kidney allograft function following transplantation.

Uromodulin (UMOD) and Osteopontin (OPN) are two proteins in the kidney commonly evaluated together in the setting of nephrocalcinosis.[7, 8] Their biological effects, however, go beyond regulation of crystal formation in the kidneys and we hypothesize that they play an important role in mal(adaptive) pathways contributing to graft outcomes. UMOD, also known as the Tamm-Horsfall protein, and osteopontin (OPN) have been shown to be synthesized by renal tubular epithelial cells and to play important roles in normal physiology and in response to renal injury.[9, 10] UMOD is produced exclusively by the kidney, primarily in the epithelial cells of the thick ascending limb,[11, 12] and has a molecular weight of around 100 kDa.[13] It is the major component of hyaline casts and the most abundant protein found in urine.[14] UMOD is also released into the renal interstitium and circulation, in small amounts that correlate with urinary secretion.[15] In the kidneys, UMOD appears to have a bidirectional role in immune modulation depending on where it is expressed. UMOD aggregates in the lumen can serve as proinflammatory ligands to help activate the innate immune response and induce an inflammatory cascade with activation of tumor necrosis factor-alpha (TNF-alpha) and granulocyte recruitment [16–18], but interstitial UMOD has anti-inflammatory functions on the renal epithelium and protective immuno-modulatory properties in the setting of experimental acute kidney injury (AKI).[19, 20] Interstitial UMOD also regulates the number and function of mononuclear phagocytes in the kidney.[20] Despite

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this bidirectional role identified pre-clinically, most epidemiological associations between UMOD and chronic kidney disease (CKD) progression show that high UMOD levels are associated with decreased risk of CKD and cardiovascular mortality.[21–23] Therefore, we hypothesize that UMOD will be associated with reduced graft failure in the deceased donor kidney transplantation setting.

OPN is a smaller protein and has a molecular weight of about 44 kDa.[24] OPN is commonly synthesized and concentrated in bone and epithelial tissues, but has also been shown to be synthesized in the thick ascending limb and by T-cells.[10] OPN may serve as a regulator in a number of metabolic and inflammatory diseases.[10] In the kidney, OPN expression is up-regulated in injury and recovery processes.[25–27] OPN has been shown to have protective effects on kidney function and long-term outcomes, such as in the setting of nephrocalcinosis and vascular calcifications.[28, 29] Hence, we hypothesize that OPN will also be associated with reduced graft failure in the deceased-donor kidney transplantation setting.

The associations of UMOD and OPN in deceased donor and long-term recipient outcomes have not been investigated. Using a multicenter, prospective cohort study of deceased donors, we determined the associations of urinary UMOD and OPN at the time of organ procurement with recipient death-censored graft failure (dcGF), and our secondary outcomes of all-cause graft failure (GF: all-cause mortality, return to dialysis, or re-transplantation). We also performed preclinical experiments to provide mechanistic insights to our identified epidemiologic associations.

Results

Donor and Recipient Characteristics

A total of 1298 donors and 2430 recipients met the inclusion criteria (Figure S1). Donors had a mean (SD) age of 41 (15) years; 784 (60%) were male and 205 (16%) were black (Table 1). Recipients had a mean age of 53 (15) years; 1492 (61%) were male and 956 (39%) were black (Table 1). Donation after neurologic determination of death occurred in 1092 (94%) donors. The most frequent comorbidities among donors were hypertension (31%), diabetes (10%), and obesity (32%). For recipients, the most common causes of end-stage kidney disease were diabetes (30%) and hypertension (26%). Mean (SD) kidney donor profile index was 48 (27). Most donor and recipient characteristics were not significantly different by UMOD or OPN tertiles (Tables S1a–S2b). Terminal serum creatinine, however, was lower with increasing UMOD tertiles but greater with increasing OPN tertiles. Cold ischemia time and the number of human leukocyte antigen mismatches were also greater with increasing tertiles of both UMOD and OPN.

Distribution of UMOD and OPN in Donor AKI

A total of 322 (25%) donors had AKI (Table 1), with the majority having stage 1 AKI (16%), followed by stage 2 (5%) and stage 3 (4%). Donor urine UMOD concentrations were significantly lower with increasing AKI stages, $p<0.001$ (Figure 1). This trend remained consistent after indexing UMOD to urine creatinine, $p<0.001$ (Table S3).

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Levels of urine OPN increased with worsening AKI severity ($p<0.001$) up to stage 2 (Figure 1) with a consistent pattern after indexing to urine creatinine, $p=0.004$ (Table S3).

Association between UMOD and OPN and Recipient Outcomes

The mean event rate (95% CI) for dcGF and GF was 33 (29.6, 36.9) and 65.7 (60.8, 71.1) per 1000 person-years, respectively, over a median (IQR) follow up time of 4.01 (2.97, 5.01) years.

Each doubling of UMOD levels in donor urine was associated with increased risk for dcGF and GF in recipients with adjusted hazard ratios [aHR (95% CI)] of 1.10 (1.01–1.19) and 1.07 (1.01–1.13), respectively, after adjustment for kidney donor risk index (KDRI), donor urine creatinine, and clinical covariates (Table 2). Tertiles of UMOD demonstrated increasing event rates of dcGF and GF, though HRs were not significant for UMOD tertiles. There were no significant interactions by donor AKI status on the relationship between UMOD and dcGF or GF.

Each doubling of donor urine OPN concentration was independently associated with decreased risk for dcGF [0.95 (0.89–1)] and GF [0.96 (0.93–1)]. A dose-response effect was observed such that the upper tertile showed a significant protective effect against GF compared to the lower tertile of donor urine OPN.

Uromodulin Osteopontin Ratio

In order to capture the opposing associations of UMOD and OPN with graft outcomes and to create a biomarker score for clinical application, we explored the ratio of UMOD to OPN urinary levels at the time of organ procurement in our training dataset. The baseline characteristics of donors and recipients in the training and test datasets are shown in Table S4a and S4b. The ratio of UMOD to OPN demonstrated independent associations in the training dataset (Table 3). In Figure 2, unadjusted Kaplan-Meier curves showed significantly lower graft survival with UMOD/OPN >3 as compared to ≤3 (log-rank $p=0.0016$). In fully adjusted models as shown in Table 3, participants with a ratio ≤3 had a 43% and 26% decreased risk of dcGF and GF, respectively. There were no significant interactions by donor AKI status on the relationship between UMOD to OPN ratio and dcGF or GF. In the test dataset, the association for GF was confirmed. The association for dcGF lost statistical significance but had a similar estimate.

UMOD and OPN Immunohistochemical Confirmation

We assessed the staining pattern of UMOD and OPN in human control kidneys compared to those showing histologic features of acute tubular injury (clinicopathologic and demographic summary shown in Table S5). In control tissues, we observed that UMOD staining (red) is limited mostly to the loop of Henle and no significant staining for OPN (teal) was observed (n=4) (Figure S2, Panel A). By contrast in deceased-donor biopsies with features of acute tubular injury, there is prominent staining of UMOD and OPN in tubular casts and injured tubules including proximal tubules and loop of Henle (n=6) (Figure S2, Panel B). Thus we confirm by immunohistochemistry that the increased expression of OPN in injured tubular segments can be observed together with UMOD in the setting of AKI.

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UMOD induces MHCII expression on macrophages

To provide mechanistic insight to our epidemiological findings, we examined the effect of a non-polymerizing truncated human UMOD on the expression of the major histocompatibility complex II (MHCII) in bone marrow-derived mouse macrophages (Figure 4). Our results show that UMOD significantly increases MHCII expression on macrophages, measured by flow cytometry.

Discussion

In this prospective deceased-donor cohort, donor urine UMOD levels decreased while OPN levels increased with increasing severity of donor AKI. UMOD was associated with increased risk of dcGF while OPN demonstrated a protective association with regard to dcGF. A ratio of UMOD to OPN ≤3 at the time of organ procurement was protective against dcGF and all-cause GF. The ratio of these two markers provides a construct that captures their bidirectional associations, which may help identify deceased-donor kidneys at the time of organ procurement that are more likely to have favorable outcomes. Based on our hypothesis, we expected urine UMOD to have protective associations with dcGF, but we found that it was associated with increased dcGF. To investigate this further, we tested the role of UMOD in MHCII expression, as chronic rejection is thought to be one of the driving causes of long-term graft failure.[30] We found that UMOD increased MHCII expression on macrophages, which may explain the identified association with increased graft failure in our cohort.

Although prior literature has shown a protective effect of UMOD on the risk of CKD, AKI and mortality[21–23, 31, 32] our findings of UMOD having an increased risk of dcGF are supported by other studies that have shown urine UMOD to be associated with increased risk of incident CKD and type 1 diabetic nephropathy [33, 34] Kidney transplantation likely presents a unique situation where the immunomodulatory properties of UMOD can cause a maladaptive response. Interstitial UMOD is known to regulate macrophage number and function in the kidney.[17, 20] Therefore, it is possible that the maladaptive role of high UMOD production in the setting of transplant could be related to its immuno-modulatory properties in the renal interstitium. Our findings suggest that UMOD can increase the expression of MHCII in macrophages, thereby enhancing their antigen presenting potential and augmenting the immunostimulatory state in the kidneys. We propose that an ongoing state of immuno-stimulation in patients with high UMOD production may negatively impact graft function, and can partially explain the increased risk of long-term graft failure associated with high UMOD levels. Lastly, it is important to note that when Uromodulin was evaluated post kidney transplantation a bimodal association was identified where lowest and highest tertiles were protective, but the middle tertile had higher risk of fibrosis and tubular atrophy.[35] Other studies have also shown improved outcomes with less GF with higher levels of serum Uromodulin post transplantation.[36, 37] Our study only evaluated pre-transplant donor urine levels of UMOD and hence it is possible that post-transplant associations and serum level associations differ from pre-transplant.

On the other hand, OPN expression has been shown to be higher in patients with recovery from AKI[29] and has also been shown to be protective against nephrocalcinosis.[28] We have

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shown that OPN is associated with decreased risk of dcGF, which supports our hypothesis. OPN has renoprotective effects through reduction of tubular cell apoptosis, regeneration and repair of tubular cells, and reduction of cell peroxide levels.[10, 25, 38]

Our cohort-based findings suggest that donor urine OPN was protective against graft failure, whereas urine UMOD was associated with graft failure, without significant interactions by AKI status. Together, the balance of donor urine UMOD and OPN captured in ratio form may not only provide more granular information on graft quality than serum creatinine, but also characterize a kidney's recovery potential after fluctuations in serum creatinine (AKI) prior to nephrectomy. In our study, a UMOD:OPN ratio ≤3 was protective against dcGF and GF. As this ratio was data-driven, we have internally validated our results with similar findings in both the derivation and the validation cohorts.

Furthermore, our biopsy findings show that OPN increases in tandem with UMOD during deceased-donor kidney injury. It is important to note that UMOD staining in casts does not translate into increased expression of UMOD in the kidney per se. It is established that in the setting of AKI, casts number increases and it is possible that increased UMOD staining reflects the increase in casts. In fact, it has been shown in pre-clinical models that UMOD expression is decreased in injury, which correlates with our findings of decreased urine UMOD in the setting of deceased donor AKI.[39]

There are several strengths to our study. The Deceased Donor Study is a large prospective cohort that includes both donor urine measurements and recipient outcomes. This unique design allows us to investigate potential tools to improve kidney allocation decisions. To date, our study is the largest adult cohort evaluating the relationship between UMOD and OPN in the setting of deceased-donor AKI and their role in recipient outcomes. Furthermore, we accounted for differing donor urine volumes and dilution by adjusting for urine creatinine in our analyses. Finally, our ratio findings were developed in a training dataset and validated in a test dataset, which suggests that our findings were not due to re-substitution bias or model-selection bias.[40]

There are also several limitations worth noting. First, both markers were measured at a single time point of organ procurement. This prevents us from assessing any post-transplant trajectories and associations with outcomes. It remains unclear how urine UMOD levels change after transplantation and what correlations post-transplant recipient levels have with pre-transplant donor UMOD levels. As with all observational studies, our study is subject to unmeasured confounding that could have affected the identified associations. Finally, although our results were internally validated and showed statistical and clinical significance, we acknowledge that external validation will be necessary to advance these findings to clinical practice.

In conclusion, our study shows moderately strong associations of UMOD and OPN with donor and recipient outcomes. A ratio of UMOD to OPN ≤3 was protective against dcGF and GF. These findings were validated in our test dataset. This ratio may be a clinically meaningful method for capturing the dynamic processes that take place in deceased-donor

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kidney transplantation and may offer a more timely and accurate way to help allocate donor kidneys than is currently available in clinical practice.

Methods

Study Population

The Deceased Donor Study (DDS) is a multicenter, observational, cohort study of deceased donors and their corresponding kidney recipients. DDS includes deceased donors in collaboration with five organ procurement organizations (OPOs): Gift of Life Donor Program, Philadelphia, PA; New Jersey Sharing Network, New Providence, NJ; Gift of Life Michigan, Ann Arbor, MI; New York Organ Donor Network, New York, NY; and New England Organ Bank, Waltham, MA. Donor urine samples were collected at the time of organ procurement from May 2010 to December 2013. Inclusion criteria were deceased donors at least 16 years of age with both admission and terminal serum creatinine. Donors were excluded if both kidneys were discarded or if they were missing urine samples.[4] Clinical variables for deceased donors were abstracted from OPO charts, and data for recipients were obtained from the Organ Procurement and Transplantation Network (OPTN). The OPTN data system includes data on all donors, wait-listed candidates, and transplant recipients in the US, submitted by the members of the OPTN, and has been described elsewhere. The Health Resources and Services Administration, U.S. Department of Health and Human Services provides oversight to the activities of the OPTN contractor. The analyses are based on OPTN data as of July 31, 2017 and may be subject to change due to future data submission or correction by transplant centers. The OPO scientific review committees and the institutional review boards for the participating investigators approved this study.

Operational Definitions of Outcome Variables

The primary outcomes of interest were donor AKI and death-censored graft failure (dcGF). Donor AKI was defined as a 50% increase in terminal serum creatinine concentration from admission or an absolute increase in serum creatinine of 0.3 mg/dL, irrespective of urine output or time from admission to terminal serum creatinine measurement. Stages of AKI were defined by Acute Kidney Injury Network criteria. Our secondary outcome of interest was all-cause GF, which was defined as all-cause mortality, return to dialysis, or re-transplantation. For the outcome of AKI, we had at least 80% power to detect an odds ratio of at least 1.48 between the 3rd and 1st tertile of a biomarker given the event rate in the 1st tertile was 34% and sample size of 430 in each group with an alpha of 5%. For the outcome of death-censored graft failure, we had at least 80% power to detect a hazard ratio of at least 1.22 per biomarker SD assuming an event rate of 13%, alpha of 0.05 and the correlation between the biomarker and other covariates was approximately 0.3. PASS v12 was used for all sample size calculations.

Measurement of UMOD and OPN

Upon transfer to the donor operating room, 10 ml of urine was obtained from the catheter tubing and then transported on ice to the OPO, where it was stored at −80°C. Samples were delivered to the Yale University biorepository monthly. Upon arrival to

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the biorepository, samples underwent a single controlled thaw, were centrifuged at 2000g for 10 minutes at 4 °C, separated into 1-ml aliquots, and immediately stored at −80 °C until UMOD and OPN measurements. UMOD and OPN were measured with the Meso Scale Discovery platform (Meso Scale Diagnostics, Gaithersburg, MD), which uses electrochemiluminescence detection combined with patterned arrays. All laboratory personnel were blinded to donor and recipient information.

Statistical Analysis

Continuous variables were reported as mean (standard deviation, SD) or median (interquartile range, IQR). Categorical variables were reported as frequencies, n (%). Differences in clinical and demographic characteristics were evaluated by the Kruskal-Wallis test or Chi-square test for continuous or categorical variables, respectively. As no clinically accepted cut-offs are available for UMOD and OPN, we evaluated the associations between these two markers and outcomes both as continuous (log_2-transformed) and categorical (tertiles) variables. We used logistic regression models to evaluate the association between each marker and donor AKI. Our logistic model for donor AKI adjusted for the following donor characteristics: age, body mass index, black race, hypertension, diabetes, stroke as the cause of death, hepatitis C serostatus, donation after circulatory determination of death status, and terminal urine creatinine. The odds ratios and 95% confidence intervals of both the univariable and multivariable models are reported.

We used Cox proportional hazard models to assess the associations of the proteins with dcGF and GF. The proportional hazards assumption was evaluated by Kolmogorov-type supremum test. The hazard ratios and 95% confidence intervals of both the univariable and multivariable models are reported. Since one donor may have one or two recipients, we estimated 95% confidence intervals using a robust sandwich covariance matrix estimator to account for intracluster dependence.[41] All inference testing was two-sided with a significance level of 0.05. Cox proportional hazards models adjusted for KDRI, urine creatinine, cold ischemia time, and the following recipient characteristics: age, black race, sex, previous kidney transplant, diabetes as the cause of end-stage kidney disease, number of human leukocyte antigen mismatches, panel reactive antibody, body mass index, and pre-emptive transplant.

We randomly divided our cohort of 2430 recipients into a training dataset and a test dataset with 1215 recipients and their corresponding donors in each dataset. In the training dataset, we explored combinations of UMOD and OPN and the association with dcGF. Given the opposing associations of UMOD and OPN with renal outcomes in prior literature,[29, 33] we evaluated the ratio of UMOD to OPN continuously (ratio of log_2-transformed UMOD and log_2-transformed OPN) and as a categorical variable (tertiles of the ratio) to assess the association of the combined repair markers with dcGF and all-cause GF. Tertile categories were derived from spline plots, and a data-driven cut-point of greater than 3 was established based on the ratio values in the third tertile. Given that there is no established ratio in the literature or a clinically established cut-off, we enhanced the validity of our results by deriving univariate and multivariate Cox proportional hazards in the training dataset and then internally validating our results in the test dataset.

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All analyses were conducted on SAS 9.4 software (SAS Institute, Cary, NC) and Stata version 14 (StataCorp LLC).

Immunohistochemical Staining and Quantification

We performed double staining for both UMOD and OPN on 11 deceased-donor kidney tissue samples from a pathology biobank (6 biopsies with acute tubular injury and 4 biopsies without acute tubular injury). Antigen retrieval was performed with citrate (pH 5.8) and endogenous peroxidase and alkaline phosphatase reactions were blocked with levamisole hydrochloride (abcam, Cambridge, MA) and PolyDetector peroxidase block (BioSB, Santa Barbara, CA) for 10 minutes. Tissue sections were incubated for 60 minutes with mouse monoclonal OPN antibody (1:200, LFMb-14, Santa Cruz Biotechnology, Inc., Dallas, Texas) and rabbit polyclonal anti-UMOD antibody (1:1000, MilliporeSigma, St. Louis, MO. Detection was performed using horseradish peroxidase polymer anti-mouse IgG with Emerald green substrate and alkaline phosphatase polymer anti-rabbit IgG with permanent red substrate (DoubleStain IHC Kit abcam, Cambridge, MA). OPN was interpreted as positive in green-stained areas, while red stain indicated UMOD positivity. Co-localization was appreciated as follows: blue - OPN expressed at higher concentrations compared to UMOD; purple - UMOD expressed at higher concentrations.

Mouse bone marrow macrophage isolation and MHCII expression assay

Truncated human tUMOD was isolated from urine of normal human donors and purified from endotoxin as described previously.[20] Bone marrow macrophages were isolated from Sv129 mice as described in Zhang, *et al.*[42] Briefly, mice were euthanized before isolating femurs and tibias. Bone marrow was flushed with cold sterile wash medium (PBS with 1% FBS) using a 25 gauge needle. Cells were grown on non-coated 100 mm dishes for six days in macrophage complete medium (RPMI 1640 with L-gluatmine (Corning 10–040-CV), 10% FBS (Gibco 10437–028), 1X penicillin/streptomycin (Gibco 15240062) and 10 ng/mL recombinant mouse M-CSF (Gibco PMC2044). Cells were harvested on day 6 by treatment with ice-cold PBS/2.5 mM EDTA, pH 8.0 and gentle scraping. Cells were replated in 6 well dishes and treated with 20 ng/ml recombinant mouse interferon gamma (Millipore Sigma IF005), tUMOD (1 ug/mL in 5% dextrose) or vehicle (5% dextrose) for 18 hours. Cells were then harvested with accutase (Biolegend 423201) and blocked with Rat anti-Mouse CD16/32 (eBioscience 50–112-9525) before staining with APC Rat anti-Mouse MHCII (eBioscience 17–5321-82). Propidium iodide was used to stain dead cells. Flow cytometry was performed on a Guava easyCyte (EMD Millipore) flow cytometer. FlowJo software was used to analyze the data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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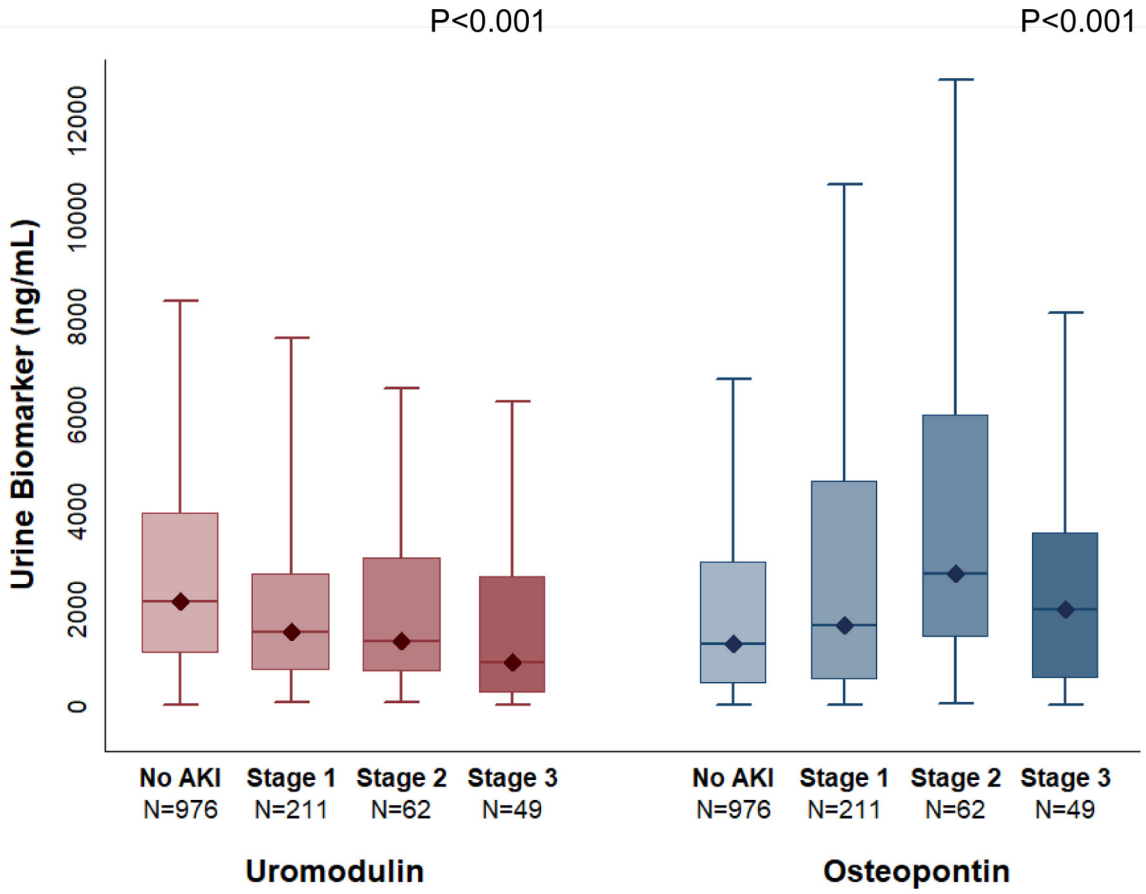

Figure 1.

Median (IQR) Levels of Donor UMOD and OPN by Donor AKI

The above box plots show that Uromodulin levels measured in the urine of deceased donors at time of organ procurements, decrease with increasing stages of AKI, whereas Osteopontin levels increase with increasing stages of AKI, although decrease slightly from stage 2 to stage 3 AKI.

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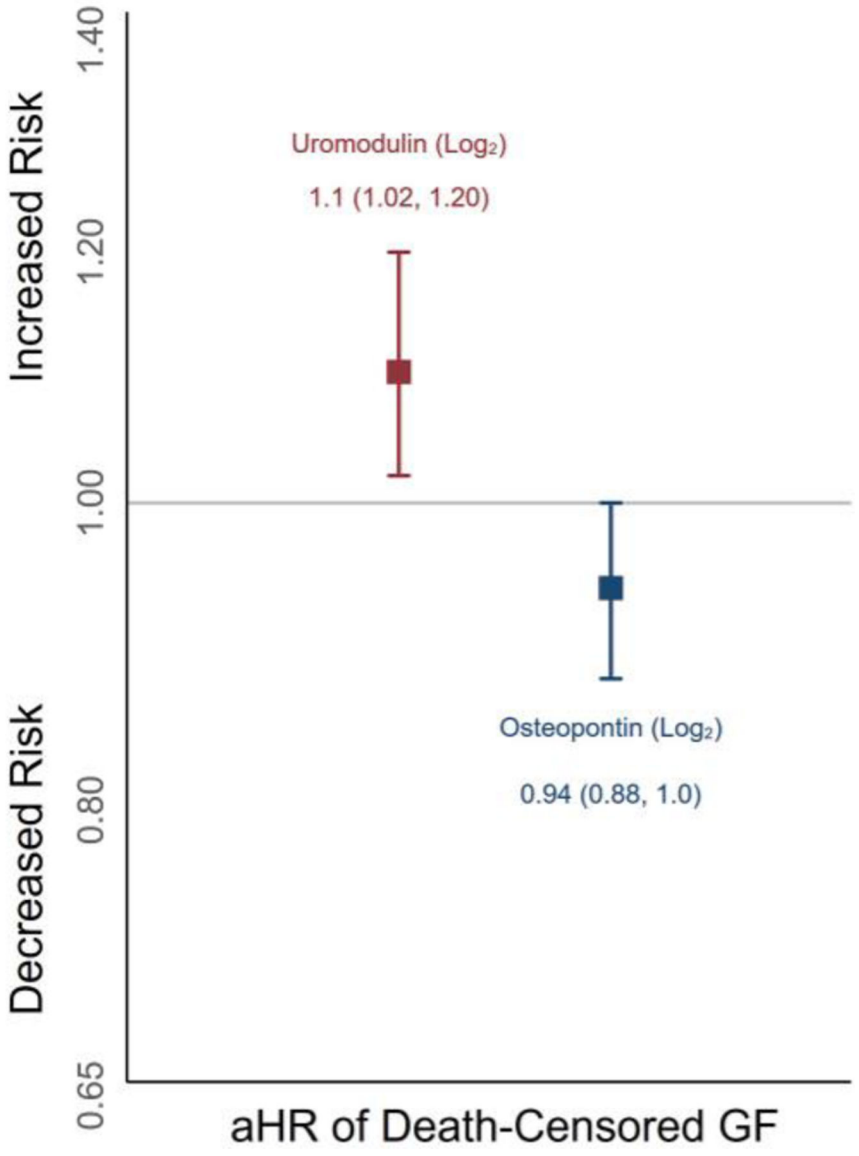

Figure 2.

Associations of UMOD and OPN with Death-Censored Graft Failure

The above figure shows the adjusted odds ratios and adjusted hazard ratios for donor AKI and death-censored graft failure. Each doubling in Uromodulin levels was associated with increased risk of death-censored graft failure. In contrast, each doubling of Osteopontin was associated with a decreased risk of death-censored graft failure.

aHR, adjusted hazard ratio; AKI, acute kidney injury; aOR, adjusted odds ratio; GF, graft failure.

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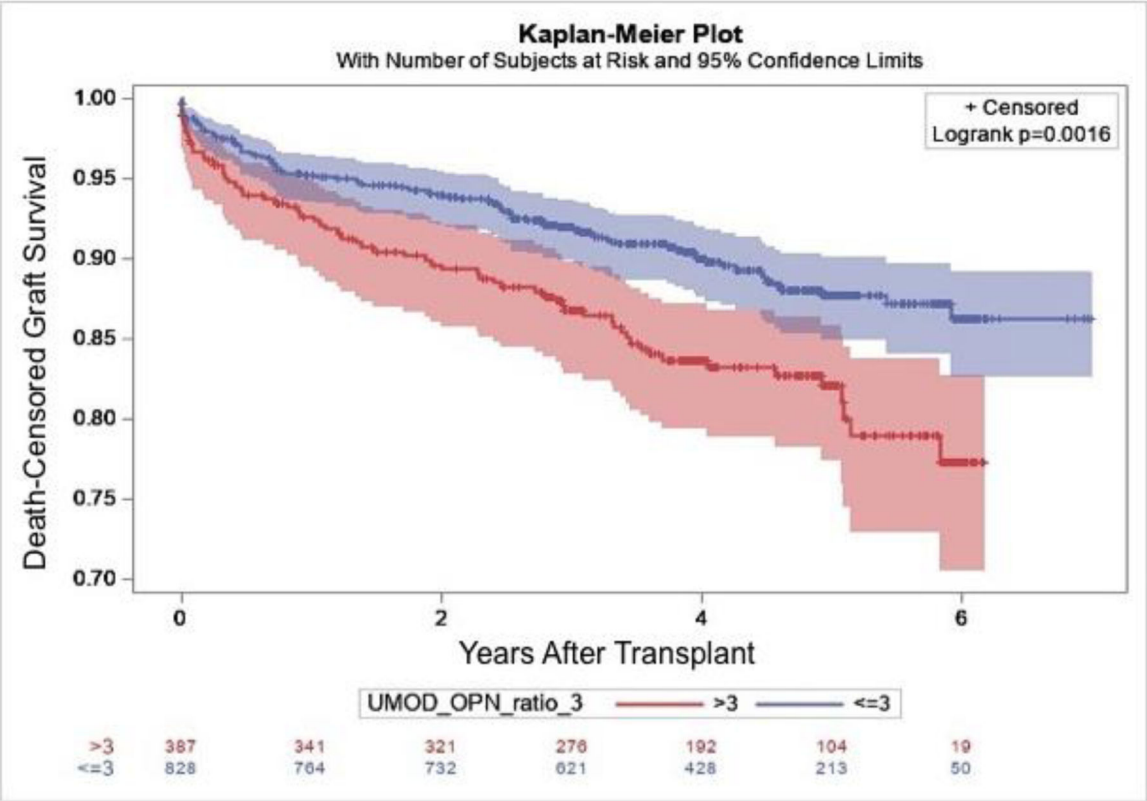

Figure 3.

Kaplan Meier Plot of Death-Censored Graft Failure by UMOD/OPN Ratio in the Training Dataset

The above survival curve shows that deceased donor kidneys with lower UMOD to OPN ratio have better graft survival with a log-rank p-value of 0.0016. The numbers below in red and blue show the population at risk at each event time with red representing donor urine with UMOD to OPN ratio >3 and blue representing UMOD to OPN ratio ≤3, respectively. Primary non-function was included as survival time of zero.

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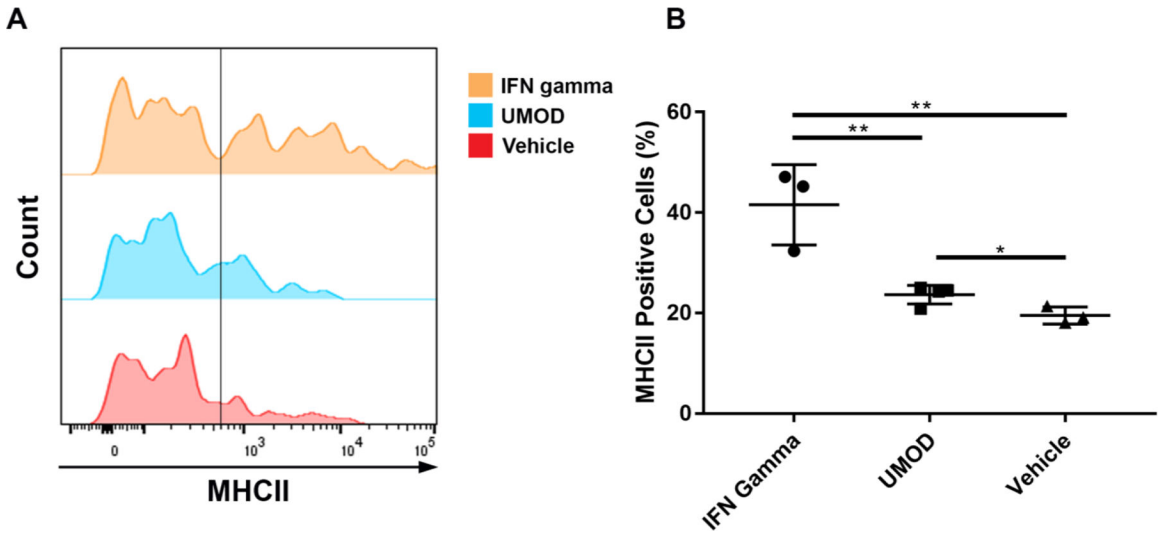

Figure 4.

Effect of Uromodulin on the expression of MHCII in bone marrow derived mouse macrophages

Panel A shows representative histograms for MHCII expression by flow cytometry in macrophages treated with vehicle, UMOD or Interferon gamma (IFN gamma), the latter used as a positive control. In B, scatter plots showing all the replicates in each condition are shown. * $p<0.05$; ** $p<0.01$

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Table 1.

Donor and Recipient Characteristics in Overall Cohort

Donor Characteristics	N=1298	Recipient Characteristics	N=2430
Age, years	41.44 (14.53)	Age, years	52.91 (14.83)
Male	784 (60%)	Male	1492 (61%)
Black Race	205 (16%)	Black Race	956 (39%)
Hispanic Race	171 (13%)	Hispanic race	279 (11%)
Body mass index, kg/m^2	28.42 (7.23)	Body mass index, kg/m^2)	28.04 (5.76)
Hypertension	399 (31%)	Cause of ESKD	
Diabetes	130 (10%)	Unknown/other	496 (20%)
Cause of Death		Diabetes	726 (30%)
Head Trauma	396 (31%)	Hypertension	643 (26%)
Anoxia	425 (34%)	Glomerulonephritis	391 (16%)
Stroke	427 (34%)	Graft Failure	174 (7%)
Other	18 (1%)	ESKD duration (months)	45.85 (38.06)
Hepatitis C	48 (4%)	Preemptive Transplant	274 (11%)
DCD including DND	246 (19%)	Previous Kidney Transplant	315 (13%)
DCD	206 (16%)	Recipient Transfusions	438 (18%)
KDPI based on KDRI	48.23 (27.34)	Candidate most recent PRA	21% (35%)
Days from admission to death	5.07 (6.7)	Recipient panel reactive antibody	
Admission SCr, mg/dL	1.1 (0.61)	0%	1545 (64%)
Terminal SCr, mg/dL	1.17 (0.85)	1–20%	178 (7%)
AKI Stage		21–80%	326 (13%)
No AKI	976 (75%)	>80%	381 (16%)
Stage 1	211 (16%)	Kidney Biopsied	1117 (46%)
Stage 2	62 (5%)	Kidney Pumped	952 (39%)
Stage 3	49 (4%)	Cold ischemia Time, hours	15.29 (7.1)
Kidneys discarded		HLA mismatch level	4.21 (1.52)
0	1132 (87%)		
1	166 (13%)		

Values are means (SD) or n (%).

Abbreviations: AKI, acute kidney injury; BMI, body mass index; ESKD, end-stage renal disease; DCD, donation after cardiac determination of death; DND, donation after neurologic determination of death; HLA, human leukocyte antigen; KDRI, kidney donor risk index; KDPI, kidney donor profile index; PRA, panel reactive antibodies; SCr, serum creatinine.

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Table 2.

Association of Donor UMOD and OPN with Donor AKI

Urine Markers	Range of Values (ng/mL)	Donors with AKI n (%)	Unadjusted OR (95% CI)	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)
Uromodulin					
Log_2 (n=1298)		322 (25%)	0.77 (0.71, 0.84)	0.76 (0.7, 0.84)	0.72 (0.65, 0.8)
Tertile 1 (n=439)	(31, 1248)	148 (34%)	1 (ref)	1 (ref)	1 (ref)
Tertile 2 (n=434)	(1248, 3052)	92 (21%)	0.53 (0.39, 0.72)	0.55 (0.4, 0.75)	0.5 (0.42, 0.68)
Tertile 3 (n=425)	(3065, 40,400)	82 (19%)	0.47 (0.34, 0.64)	0.48 (0.35, 0.67)	0.41 (0.29, 0.59)
Osteopontin					
Log_2 (n=1298)		322 (25%)	1.14 (1.06, 1.22)	1.15 (1.07, 1.23)	1.18 (1.09, 1.28)
Tertile 1 (n=433)	(53, 808)	92 (21%)	1 (ref)	1 (ref)	1 (ref)
Tertile 2 (n=433)	(809,2445)	99 (23%)	1.1 (0.8, 1.52)	1.17 (0.84, 1.63)	1.22 (0.87, 1.72)
Tertile 3 (n=432)	(2448,46,100)	131 (30%)	1.61 (1.19, 2.2)	1.71 (1.24, 2.35)	1.86 (1.3, 2.67)

[a]Model 1 includes donor age (years), BMI, black race, history of hypertension, history of diabetes, stroke as cause of death, hepatitis C serostatus, and donation after circulatory determination of death status.

[b]Model 2 includes all covariates in Model 1 plus urine creatinine.

Complete case analysis was completed since there were no missing data.

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Table 3.

Association of Donor UMOD and OPN with Risk of Death-Censored Graft Failure and All-Cause Graft Failure

		Hazard Ratio (95% Confidence Interval)	
	Mean (95% CI) Event Rate per 1000 Person Year	**Unadjusted**	**Adjusted[a]**
Death-Censored Graft Failure (dcGF)			
Uromodulin			
Log2 (n=2430)	33 (29.6, 36.9)	1.11 (1.03, 1.19)	1.1 (1.02, 1.2)
Tertile 1 (n=810)	28.6 (23.2, 35.2)	1 (ref)	1 (ref)
Tertile 2 (n=810)	33.9 (28.1, 41)	1.19 (0.9, 1.57)	1.12 (0.84, 1.5)
Tertile 3 (n=810)	36.4 (30.4, 43.6)	1.27 (0.96, 1.67)	1.2 (0.89, 1.62)
Osteopontin			
Log2 (n=2430)	33 (29.6, 36.9)	0.95 (0.89, 1)	0.94 (0.88, 1)
Tertile 1 (n=810)	38.6 (32.2, 46.2)	1 (ref)	1 (ref)
Tertile 2 (n=810)	29.9 (24.4, 36.5)	0.78 (0.59, 1.02)	0.82 (0.61, 1.08)
Tertile 3 (n=810)	31 (25.5, 37.7)	0.8 (0.62, 1.05)	0.76 (0.56, 1.04)
All-Cause Graft Failure (GF)			
Uromodulin			
Log 2 (n=2430)	65.7 (60.8, 71.1)	1.06 (1, 1.12)	1.07 (1.01, 1.13)
Tertile 1 (n=810)	60.1 (52.1, 69.4)	1 (ref)	1 (ref)
Tertile 2 (n=810)	66.9 (58.5, 76.6)	1.11 (0.91, 1.36)	1.12 (0.91, 1.37)
Tertile 3 (n=810)	70 (61.4, 79.8)	1.13 (0.93, 1.37)	1.16 (0.95, 1.43)
Osteopontin			
Log2 (n=2430)	65.7 (60.8, 71.1)	0.96 (0.93, 1)	0.95 (0.91, 1)
Tertile 1 (n=810)	71.8 (62.9, 82)	1 (ref)	1 (ref)
Tertile 2 (n=810)	64.7 (56.5, 74.1)	0.89 (0.74, 1.08)	0.86 (0.7, 1.05)
Tertile 3 (n=810)	61 (53, 70.1)	0.83 (0.68, 1)	0.77 (0.61, 0.96)

[a]Adjusted for urine creatinine, KDRI, and the following clinical covariates: cold ischemia time (22 missing), recipient age (years), race, sex, prior kidney transplant, diabetes as the cause of end-stage kidney disease, number of human leukocyte antigen mismatches, panel reactive antibody (%), body mass index (1 missing), and pre-emptive transplant

There were no significant interactions by donor AKI status in the relationship between UMOD and dcGF and GF.

Complete case analysis was completed since missing data was rare. Biomarker measurements and outcomes were available in all recipients. Covariate data was complete except for cold ischemia time (0.9% missing), HLA mismatch (0.2% missing) and BMI (0.04% missing).

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Table 4.

Association of Donor UMOD to OPN ratio with Risk of All-Cause Graft Failure and Death-Censored Graft Failure in the Training and Test Data Set

Ratio of UMOD to OPN	Total N	Mean Event Rate (95% CI), per 1000 person year	Unadjusted HR (95%CI)	Adjusted[a] HR (95% CI)	Mean Event Rate (95% CI), per 1000 person year	Unadjusted HR (95% CI)	Adjusted[a] HR (95% CI)
Training Data Set							
		Death Censored Graft Failure			**All Cause Graft Failure (GF)**		
> 3	387	44.6 (35.0, 56.9)	1 (ref)	1 (ref)	76.8 (63.9, 92.5)	1 (ref)	1 (ref)
≤ 3	828	26.5 (21.5, 32.7)	0.60 (0.43, 0.83)	0.57 (0.41, 0.80)	58.4 (50.7, 67.3)	0.76 (0.60, 0.96)	0.73 (0.57, 0.93)
Test Data Set							
		Death Censored Graft Failure			**All Cause Graft Failure (GF)**		
> 3	385	43.1 (33.6, 55.1)	1 (ref)	1 (ref)	85.4 (71.7, 101.8)	1 (ref)	1 (ref)
≤ 3	830	29.9 (24.6, 36.5)	0.69 (0.51, 0.96)	0.73 (0.52, 1.02)	59.3 (51.5, 68.2)	0.69 (0.55, 0.87)	0.70 (0.56, 0.88)

Complete case analysis was completed since missing data was rare. Biomarker measurements and outcomes were available in all recipients. Covariate data was complete except for cold ischemia time (0.9% missing), HLA mismatch (0.2% missing) and BMI (0.04% missing).