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Deceased-Donor Acute Kidney Injury and Acute Rejection in Kidney Transplant Recipients: A Multicenter Cohort

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PII: S0272-6386(22)00920-9

DOI: https://doi.org/10.1053/j.ajkd.2022.08.011

Reference: YAJKD 57780

To appear in: *American Journal of Kidney Diseases*

Received Date: 13 April 2022

Accepted Date: 2 August 2022

Please cite this article as: Reese PP, Doshi MD, Hall IE, Besharatian B, Bromberg JS, Thiessen-Philbrook H, Jia Y, Kamoun M, Mansour SG, Akalin E, Harhay MN, Mohan S, Muthukumar T, Schröppel B, Singh P, Weng FL, Parikh CR, Deceased-Donor Acute Kidney Injury and Acute Rejection in Kidney Transplant Recipients: A Multicenter Cohort, *American Journal of Kidney Diseases* (2022), doi: https://doi.org/10.1053/j.ajkd.2022.08.011.

Deceased-Donor AKI and Acute Rejection in Kidney Transplant Recipients

Setting & Participants

Observational cohort study

13 transplant centers in US

2010-2013

862 deceased donors for 1,137 kidney recipients

- Mean recipient age: 54 ± 13 years
- 82% received anti-thymocyte globulin

Methods

Measured concentrations of IL-18, KIM-1, and NGAL in deceased donor urine at organ procurement

Recorded treatment and outcome data in kidney recipients

Ascertained outcomes in the first post-transplant year

Results

No significant association with primary outcome of rejection and graft failure

sHR for highest vs lowest tertile

- **IL-18:** 0.76 (95% CI, 0.45-1.28)
- **KIM-1:** 1.20 (95% CI, 0.69-2.07)
- **NGAL:** 1.14 (95% CI, 0.71-1.84)

No significant association between donor urinary biomarkers and secondary outcome of rejection, graft failure, and *de novo* DSA (measured at 5 centers)

CONCLUSION: In a large cohort, donor injury biomarkers were neither associated with graft failure and rejection, nor with a secondary outcome that included *de novo* DSA.

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@AJKDonline | DOI: 10.1053/j.ajkd.2022.08.011

AJKD AMERICAN JOURNAL OF KIDNEY DISEASES

Deceased-Donor Acute Kidney Injury and Acute Rejection in Kidney Transplant Recipients: A Multicenter Cohort

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ABSTRACT

Rationale & Objective: Donor acute kidney injury (AKI) activates innate immunity, enhances HLA expression in the kidney allograft, and provokes recipient alloimmune responses. We hypothesized that injury and inflammation manifested in deceased-donor urine biomarkers would be associated with higher rates of biopsy-proven acute rejection (BPAR) and allograft failure after transplantation.

Study Design: Prospective cohort.

Setting & Participants: 862 deceased donors for 1137 kidney recipients at 13 centers.

Exposures: We measured concentrations of IL-18, kidney injury molecule-1 (KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL) in deceased donor urine. We also used the Acute Kidney Injury Network criteria to assess donor clinical AKI.

Outcomes: The primary outcome was a composite of BPAR and graft failure (not from death). A secondary outcome was the composite of BPAR, graft failure, and/or *de novo* donor specific antibody (DSA). Outcomes were ascertained in the first post-transplant year.

Analytical Approach: Multivariable Fine-Gray models with death as a competing risk.

Results: Mean recipient age was 54±13 years and 82% received anti-thymocyte globulin. We found no significant associations between donor urinary IL-18 (subdistribution hazard ratio [sHR] for highest vs. lowest tertile 0.76; 95% CI 0.45, 1.28), KIM-1 (sHR 1.2; 95% CI 0.69, 2.07) or NGAL (sHR 1.14; 95% CI 0.71, 1.84) and the primary outcome. In secondary analyses, we detected no significant associations between a) clinically-defined AKI and the primary outcome, or between b) donor biomarkers and the composite outcome of BPAR, graft failure and/or *de novo* DSA.

Limitations: BPAR ascertained through for-cause biopsies, not surveillance biopsies.

Conclusions: In a large cohort of kidney recipients that were almost all induced with thymoglobulin, donor injury biomarkers were neither associated with graft failure and rejection, nor with a secondary outcome that included *de novo* DSA. These findings provide some reassurance that centers can successfully manage immunological complications using deceased-donor kidneys with AKI.

Keywords:
Kidney transplantation
Acute kidney injury
Deceased organ donation
Biomarkers
Graft failure

Plain-Language Summary

Many US patients wait years for kidney transplant because of a shortage of good-quality donated kidneys. One way to relieve this problem is to transplant kidneys that experienced inflammation and injury in the deceased donor before transplantation. We measured the level of kidney injury in the urine of 862 donors. We then studied the clinical outcomes for the 1137 adult recipients of kidneys transplanted from those donors. Compared to recipients of kidneys from donors with less injury, the recipients of injured kidney transplants did not experience higher rates of a combined outcome of rejection or failure of the transplant. These results provide evidence that transplant centers can successfully manage transplantation using injured kidneys from deceased donors.

Introduction

Deceased organ donors often experience acute kidney injury (AKI) either due to the circumstances of death, such as trauma or anoxia, or due to complications of subsequent treatment. Unfortunately, approximately one-third of kidneys from deceased donors with AKI are discarded, a higher rate than donors without AKI.[1, 2] The risk of immunological complications associated with transplanting AKI kidneys is unknown. AKI causes tissue inflammation through multiple mechanistic pathways, such as complement activation (e.g., the MB-lectin pathway) and enhanced expression of toll-like receptors (TLR) including TLR-2 and TLR-4, which are present in renal tubular epithelial cells.[3] M2 macrophages and regulatory T-cells play a direct role in guiding the response to inflammation and repair following AKI.[4] Given these inflammatory pathways, we hypothesized the recipients of AKI kidneys would experience elevated rates of acute rejection, both cellular and antibody, and formation of *de novo* donor specific antibody (DSA).

The “Injury Hypothesis” was proposed over 20 years ago (with subsequent modifications) and states that oxidative stress and injury to the kidney at procurement and transplantation variably activate innate immunity in the allograft, which affects alloimmune responses and elevates the risk of rejection.[5] Classically, this injury pathway would be expected to provoke acute cellular rejection (ACR) via alloreactive T-cells. However, B-cell responses may be affected concurrently and promote acute antibody-mediated rejection (AMR) via DSA. AMR may carry a worse prognosis than ACR, involves treatments that have not been extensively tested in clinical trials, and may cause chronic immunological injury and fibrosis.[6]

To assess outcomes using AKI kidneys, we assembled a multi-center, prospective cohort that included testing deceased-donor urine for injury biomarkers and detailed chart review of recipient outcomes (the Deceased Donor Study) including biopsies. We identified AKI using both conventional serological definitions that rely on changes in serum creatinine as well as characterizing injury using sensitive urinary biomarkers including IL-18, KIM-1, and NGAL. We showed that AKI defined using serum creatinine (corresponding to ≥Stage 2 Acute Kidney Injury Network [AKIN] criteria) was present in approximately 9% of deceased-donor kidneys and that many additional donors had elevated concentrations of injury biomarkers. Our group and others have demonstrated that recipients of AKI kidneys commonly experience delayed graft function (DGF);[7] Donor urinary biomarkers that are generated in the setting of AKI, such as NGAL, are associated with DGF in the recipient. Nonetheless, longer-term graft survival and graft function for kidneys with AKI are comparable to kidneys without AKI.[8-11] Some uncertainty remains about whether kidneys with severe, AKIN Stage 3 injury in the donor also have good long term outcomes after transplantation.[2]

We leveraged the detailed immunological data in the Deceased Donor Study to examine whether donor kidney injury and inflammation, manifested through urinary biomarkers, were associated with allograft failure and rejection. For the subset of centers with clinical protocols for routine post-transplant assessment of DSA, we developed a study protocol to harmonize adjudication of a composite outcome that included *de novo* DSA within 1 year after transplant.

Methods

The Deceased Donor Study (ClinicalTrials.gov Identifier: NCT01848249) is an observational cohort study of deceased donors with subsequent prospective data extraction from the medical records of the recipients of kidney transplants from those donors.[1, 12-17] Briefly, five participating organ procurement organizations (OPOs) enrolled donors between May 2010 and December 2013. Each OPO utilized set protocols for research authorization and donor management. Clinical variables were abstracted from OPO donor charts, and extensive chart reviews were performed for the subset of recipients ≥16 years at 13 participating transplant centers who received kidneys from enrolled donors. Trained site coordinators reviewed prospectively collected medical records and recorded detailed recipient data. Key outcomes including DGF (any dialysis in the week after transplant) and allograft biopsy results were reviewed by site principal investigators. The data coordinating center validated chart abstractions to confirm data accuracy (**Item S1**). The study also used some data from the Organ Procurement and Transplantation Network (OPTN). The OPTN data system includes data on all donor, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN), and has been described elsewhere. The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN contractor.

The OPO scientific review board approved the study and authorization for research was obtained from the surrogates of the deceased donors. The institutional review boards for participating investigators approved the study and waived informed consent for transplant recipients. The clinical and research activities being reported are

consistent with the Principles of the Declaration of Istanbul as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.[18] All clinical investigators abided by the Ethical Principles for Medical Research Involving Human Subjects as outlined in the Declaration of Helsinki.

Outcomes

The primary outcome was a composite of graft failure (not due to death) or biopsy-proven acute rejection in the first post-transplant year. Kidney biopsies, pathology interpretation and treatment for rejection were performed per each center's local protocol. In a secondary analysis, we examined the composite of graft failure, rejection or *de novo* DSA within the first year among a subcohort of five of the participating centers that screened recipients for *de novo* DSA; we did not include centers that only measured DSA due to clinical concern for rejection.

For the binary outcome of *de novo* DSA, each center applied the criterion of mean fluorescence intensity (MFI) ≥1000. For patients with pre-transplant DSA, we also categorized as a positive outcome a patient with MFI ≥1000 *and* ≥50% higher than the pre-transplant DSA MFI. MFI was defined as antibody reactivity to the specific donor human leukocyte antigen (HLA) allele, where allele level typing was available. Where necessary, the center would choose the highest MFI associated with an HLA epitope – even if the epitope was shared among several HLA alleles, one of which corresponded to the donor HLA allele. Importantly, each transplant center followed their own clinical protocol for kidney biopsies and DSA screening (**Item S2**).

Notably, we restricted outcomes to one year, because rejection and DSA development beyond 12 months would be less likely to be associated with donor injury and more likely caused by recipient clinical issues such as nonadherence.

Donor urine injury biomarker data

The primary exposure was donor urinary concentrations of IL-18 (pg/mL), KIM-1 (pg/mL), and NGAL (ng/dL). Prior to organ procurements, 10 mL of fresh donor urine was collected using an indwelling urinary catheter tube, transferred on ice, and then frozen. The urine was stored at -80 degrees Celsius until monthly shipments to the central study biorepository. Biomarker measurements are described thoroughly in the supplement (**Item S3**) and in previous work.[23]

Statistical analysis

The supplement (**Item S4**) includes details about the calculation of variables. We calculated descriptive statistics as means ± standard deviation, medians (interquartile range), or frequencies (percentages). Donor, transplant, and recipient characteristics were compared by the primary outcome using Kruskall-Wallis or Chi-Square tests. Because these comparisons were by recipient outcome, we assessed donor characteristics at the level of the kidney for these analyses. We then fit a multivariable Fine-Gray regression model to determine the subdistribution hazards ratio (sHR) for donor injury biomarkers and the primary outcome, with death as a competing risk. Donor injury biomarkers were both modeled continuously after a log base 2 transformation and as tertiles.

We used Kolmogorov-type supremum tests to evaluate proportional hazards assumptions. We accounted for the cluster effect of kidneys from the same donor going to two recipients using robust sandwich estimates. In the primary analysis, we adjusted for variables available at organ offer and collected by OPOs: the KDRI; the transplant variables cold ischemia time (hours) and number of human leukocyte antigen (HLA) mismatches; and the following

recipient variables: age (years), sex, Black race, previous kidney transplant, cause of end-stage kidney disease, percent panel reactive antibody (PRA), body mass index [BMI], and preemptive transplantation[10, 19]. In the analysis of the secondary outcome (that included *de novo* DSA), we also adjusted for pre-transplant DSA (a binary exposure). Final models also adjusted for donor urinary creatinine concentrations.

Exploratory analyses

We assessed whether the following variables modified the relationship between donor injury biomarkers and the primary outcome: KDPI (cut-off: 85%), donation after circulatory determination of death (DCD), kidney machine perfusion, cold ischemia time (median value cut-off: 14 hours), DGF, donor-recipient sex combinations,[17, 20] or donor-recipient race combinations.[21, 22] Each of these Fine-Gray models used the same covariates as the primary analysis with tests for interaction between the donor biomarker and the potential modifier.

We also fit Fine-Gray models and examined the association between donor biomarkers and the outcome of BPAR only. We then fit a Cox regression model to examine the association between donor biomarkers and the composite outcome of BPAR, graft failure, or death. Covariates were the same as for the donor biomarker models for the primary outcome. We fit Fine-Gray models to examine the association of donor clinical AKI defined as AKIN Stage 2 or greater and the primary outcome. Covariates were the same as for the donor biomarker models for the primary outcome, except that we did not adjust for urinary creatinine concentration. Finally, we examined the associations of donor clinical AKI and the outcome of BPAR only, and donor clinical AKI and the composite outcome of BPAR, graft failure, or death.

Power

We evaluated the statistical power by examining the association of biomarkers (highest vs. lowest tertile) within each outcome. We assumed an alpha of 5%, power of 80%, that the hazard ratio is constant throughout the study and that Cox proportional hazards regression models were used.[23, 24] For the primary outcome, we estimated the power to detect a hazard

ratio of at least 0.56 (within the cohort of 1137 recipients). For the secondary outcome of BPAR, graft failure, or DSA, a hazard ratio of at least 0.47 (within the cohort of 422 recipients).

We used SAS 9.4 for Windows (SAS Institute, Cary, NC). All statistical tests and confidence intervals were two-sided with a significance level of 0.05.

Results

A shown in **Figure 1**, the primary cohort comprised 1137 deceased-donor kidney transplant recipients at 13 centers.

Table 1a shows that the mean recipient age was 53.7 years (+/-13.3) and 61% were male. Fourteen percent had previously received kidney transplants and 15% had estimated PRA >80%. Eighty-two percent of recipients received rabbit anti-thymocyte globulin induction therapy, 15% received basiliximab and 3% received alemtuzumab. **Table S1** provides additional detail about immunosuppression. Compared to recipients who did not experience the primary composite outcome of rejection or allograft failure, recipients who did experience the outcome were more likely to be Black race (57% vs. 45%, p=0.003), have prior transplants (19% vs. 13%, p=0.04), and calculated panel reactive antibody titers (cPRA) above 80% (21% vs. 14%, p=0.05 [p-value for the association with all 4 levels of PRA]), but they were less likely to be discharged from the transplant hospitalization on tacrolimus (89% vs. 97%, p<0.001) and mycophenolate (93% vs. 97%, p=0.023). A total of 37% of recipients experienced DGF. Recipients who experienced the primary outcome were also more likely to have delayed graft function (54% vs. 35% for those without the primary outcome, p<0.001).

Table 1b shows that for the deceased kidney donors, mean (SD) terminal serum creatinine was 1.21 (0.93) mg/dL and 19% were DCD. When categorized by AKIN

stages, 73% of the kidneys came from donors with no AKI, 16% from donors with Stage 1 AKI, 6% from donors with Stage 2 AKI, and 5% from donors with Stage 3 AKI.

Figure 1 shows that 159 recipients (14%) experienced the primary composite of graft failure or BPAR during the first year. One hundred seven met the primary outcome due to BPAR. **Table S2** shows time-to-event. A total of 77 of BPAR episodes were ACR only, 8 were AMR only, 12 were both ACR and AMR, and 10 could not be definitively classified (**Table S3**).

Figure 2 shows distributions of donor urine IL-18, KIM-1, and NGAL concentrations. We found no significant association between urinary injury biomarkers and the primary outcome in multivariable analyses. In the fully-adjusted models with highest tertile vs. lowest tertile biomarker concentrations, the sHRs were 0.76 (95% CI 0.45, 1.28) for IL-18, 1.2 (95% CI 0.69, 2.07) for KIM-1, and 1.14 (95% CI 0.71, 1.84) for NGAL (**Table 2**).

Secondary and exploratory analyses

A total of 422 recipients at 5 centers comprised the subcohort with DSA screening (**Figure S1**). Fifty-four (13%) had pre-transplant DSA. By one year, 85 recipients (20%) experienced the composite outcome of graft failure, acute rejection and/or *de novo* DSA. Thirty-eight experienced the rejection outcome, 35 the *de novo* DSA outcome, and 12 the graft failure outcome. **Table S4** shows details about *de novo* DSA. Twelve recipients died by one year. We found no significant association between urinary injury biomarkers and the secondary composite outcome. In the fully-adjusted models with highest tertile vs. lowest tertile biomarker concentrations, the sHRs were

0.81 (95% CI 0.42, 1.56) for IL-18, 0.9 (95% CI 0.43, 1.87) for KIM-1, and 0.66 (95% CI 0.34, 1.29) for NGAL (**Table 3**).

Tables S5 & S6 show exploratory analyses of effect modification. DCD status modified the association of urinary NGAL with rejection or allograft failure. As shown in **Tables S7 & S8**, donor urinary biomarkers were neither significantly associated with the outcome of BPAR nor with a composite of BPAR, graft failure, or death.

Donor AKI, defined using the AKIN scale, was also not associated with the primary or secondary outcomes or with the outcomes of exploratory analyses (**Tables S9 – 12**).

Discussion

In this large and well-phenotyped cohort, we found no association between donor kidney injury and inflammation biomarkers and a composite outcome of graft failure and acute rejection. In a subcohort, we also found no association between these biomarkers and a composite outcome that also included DSA. A secondary analysis also detected no association between clinical AKI and the primary outcome. These findings contradict our hypothesis. We propose that donor AKI may have provoked inflammation in the allograft, but contemporary immunosuppression may have been sufficient to ameliorate immunological consequences of inflammation after transplantation. Taken together with other studies, this analysis provides new evidence that transplant centers can successfully manage complications and achieve good outcomes using AKI kidneys.[8-10, 17]

Deceased-donor kidneys with AKI are frequently discarded due to concerns about early clinical complications such as primary nonfunction and longer-term risks of allograft fibrosis.[1] Yu et al. examined kidney non-procurement among deceased donors in the US from 2000 to 2018. Compared to donors with terminal creatinine <1.00 mg/dL, those with values between 1.00-1.49 mg/dL and between 1.50-2.00 mg/dL (for AKI or any reason) were 48% and 300% more likely to have no kidneys procured, respectively.[25] It is clear that donor AKI increases the risk of recipient DGF.[1, 11] However, studies from diverse data sources have demonstrated that recipients of AKI kidneys still usually experience longer-term graft survival and allograft function similar to kidneys without AKI.[8, 9, 11, 17, 26] For example, Sonnenberg et al. examined a national US cohort of recipients of kidneys in which donor AKI was ongoing at procurement (terminal creatinine >1.5 mg/dL); one-third of these kidneys met criteria for AKI Stage 3. All-cause graft failure rates by 3 years were 15.5% for recipients of AKI vs. 15.1% for recipients of non-AKI kidneys. In multivariable adjustment, AKI kidneys were associated with only slightly higher risk of all-cause graft failure (aHR 1.05, 95% CI:1.01-1.09).[9] Prior studies from our DDS cohort examined AKI using both creatinine-based criteria and donor urinary biomarkers, which can detect subclinical AKI located in the distal tubule or other compartments of the nephron.[17] We found that higher donor NGAL was associated with recipient DGF (highest vs. lowest NGAL tertile relative risk 1.21; 95% CI 1.02, 1.43). Yet, analyses of 6-month recipient eGFR revealed that NGAL and liver fatty acid binding protein were associated with only modestly lower eGFR, and this association was restricted to recipients without DGF.[10] While a study from the United Kingdom reported higher primary nonfunction rates for Stage 3 AKI kidneys (9% vs. 4%, p=0.04) and

advised caution about accepting these kidneys,[2] those results contrast with findings from multiple other single and multi-center studies that have described favorable outcomes after kidney transplantation with AKI kidneys.[9, 11, 17]

The present analysis provides fresh data by focusing on immunological outcomes of acute rejection and *de novo* DSA. We suggest potential explanations for the lack of association between donor injury biomarkers and our composite outcome. First, many scientific insights related to HLA upregulation due to ischemic injury were derived in the ischemia-reperfusion setting at implantation and may not apply to the earlier event of donor AKI.[5, 27] Indeed, we previously made the observation that among kidney transplant recipients with DGF, recipients of kidneys from donors with elevated injury markers actually experienced *better* 6-month graft function vs. recipients of kidneys with low levels of injury. As a result, our group speculated that donor AKI might provoke ischemic preconditioning and upregulation of molecular mechanisms that protect against ischemia-reperfusion.[10] Next, we note that eighty-two percent of recipients received anti-thymocyte globulin and nearly all received tacrolimus and mycophenolate. This regimen may have been sufficient to mitigate immunological responses caused by AKI.

We acknowledge limitations. It is possible that an association between AKI and subclinical rejection exists but was undetected because of limited power or because surveillance biopsies were not part of center protocols. We also did not measure novel genetic biomarkers of rejection such as cell-free DNA or others that may reflect gene expression. On the other hand, our findings suggest that even if AKI caused subclinical rejection, the clinical consequences were limited, perhaps due to the robust

immunosuppression regimen. From that perspective, we emphasize that our results do not need to be interpreted as contradicting the "Injury Hypothesis."[5] Second, it is possible that centers only accepted AKI kidneys with otherwise favorable characteristics. We acknowledge that subsequent studies, for instance with a higher proportion of AKIN Stage 3 kidneys, might find an association between severe AKI and risk of recipient rejection. Nonetheless, we adjusted for a wide range of characteristics relevant to immunological outcomes, including HLA mismatch, PRA, and recipient age. We call attention to a recent study using this cohort in which donor AKI was associated with reduced risk of BK virus infection and BK nephropathy-associated graft failure.[28] This finding suggests the possibility that specific and, thus far undefined, pathways of immunological activation in a donor AKI kidney might be protective against viral infection. The present study also has the limitation that DSA assessment took place at each center's laboratory. However, all five centers in the subcohort used the same screening platform and single antigen beads to characterize DSA. The investigators then applied uniform criteria to the binary outcome of *de novo* DSA. An additional limitation is that all participating centers were academic medical centers. We also did not adjust for induction therapy or perfusion pumping, because of concerns about confounding-by-indication for kidneys at risk of injury. We also emphasize the study's strengths in that the population was large, ethnically diverse, and the kidney transplant recipients were treated with the most common immunosuppressive regimens used nationally and experienced outcomes such as rejection and graft failure at rates similar to the national experience.[29]

In this multi-center study with close follow-up of recipients, donor injury biomarkers were neither associated with the primary outcome of graft failure and rejection, nor with a secondary outcome that included *de novo* DSA. These results should be confirmed in other cohorts. For transplant centers trying to develop greater experience with transplanting donor AKI kidneys, these findings provide initial evidence that accepting deceased-donor kidneys with AKI will not substantially increase risks of acute rejection under a regimen of robust immunosuppression.

Supplemental Material Table of Contents

Table S12: Multivariable analysis of clinically-defined donor acute kidney injury and the exploratory composite outcome of BPAR, graft failure or death

ARTICLE INFORMATION

Authors' Contributions: Research area and study design: CRP, PPR, IEH, MDD, BS; data acquisition: PPR, MDD, IEH, BB, JSB, MK, SGM, EA, MNH, SM, TM, BS, PS, FLW, CRP; data analysis and interpretation: all authors; statistical analysis: HTP, YJ. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

Support: This work was supported by the National Institutes of Health (NIH)/National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) grant R01DK93770 and grant K24DK090203 to Dr. Parikh; George M. O'Brien Kidney Center at Yale Grant P30DK079310 to Dr. Parikh; a grant to Dr. Hall by the NIH/National Center for Advancing Translational Sciences (NCATS) under award numbers UL1TR002538 and KL2TR002539. This work was supported in part by Health Resources and Services Administration contract HHSH250-2019-00001C. The funders did not have a role in study design, data collection, analysis, reporting, or the decision to submit for publication.

Financial Disclosure: The authors declare that they have no relevant financial interests. The following lists nonrelevant financial interests for authors who hold them. Dr. Bromberg acknowledges clinical research support from Novartis, CareDx, and

Natera. Dr. Weng acknowledges support as co-investigator from Merck, Novartis, Angion Biomedica, CareDx, Medeor Therapeutics, CSL Behring, and Natera. Dr. Harhay acknowledges a consultancy agreement with Nephria Bio. Dr. Reese has received grant support from Merck, AbbVie, and Gilead related to transplanting organs from HCV-infected donors; and has also consulted for VALHealth on the identification of patients with CKD. Dr. Parikh is a member of the advisory board of and owns equity in RenalytixAI, and also serves as a consultant for Genfit and Novartis. Dr. Mohan has received grant support from the Kidney Transplant Collaborative and Angion Biomedica. Dr. Schröppel acknowledges consultancy agreements with Novartis, Astellas, Amgen, Vifor Pharma, and AstraZeneka. Dr. Enver is a member of the advisory board for CareDx, Immucor, Exosome, and Takeda; has received grants support from CareDx, and Immucor; and is a principal investigator on clinical trials with Astellas, Angion, and Ampylix. All authors report receipt of NIH support (as noted in previous section, and also additional grants).

Disclaimer: The data reported here have been supplied by the United Network for Organ Sharing (UNOS) as the contractor for the OPTN. The interpretation and reporting of these data are the responsibility of the authors and in no way should be seen as an official policy of or interpretation by the OPTN or the US Government. The content is the responsibility of the authors alone and does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Peer Review: Received April 13, 2022. Evaluated by 2 external peer reviewers and a statistician, with editorial input from an Acting Editor-in-Chief (Editorial Board Member

Vinod Bansal, MD). Accepted in revised form August 2, 2022. The involvement of an Acting Editor-in-Chief to handle the peer-review and decision-making processes was to comply with AJKD's procedures for potential conflicts of interest for editors, described in the Information for Authors & Journal Policies.

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Figure legends

Figure 1: Flow chart for the primary cohort

Figure 2: Donor urinary biomarker distributions, by recipients who did and did not experience the primary composite outcome of graft failure or acute rejection

Table 1a: Recipient characteristics, by primary outcome status

Recipient Characteristics		All (N=1137)	Non-Event (Including death)* (N=978)	Composite Event** (N=159)	P
Mean age, years		53.7 (13.3)	53.7 (13.3)	53.7 (13.4)	1.0
Male		693 (61%)	597 (61%)	96 (60%)	0.9
Black race		528 (46%)	437 (45%)	91 (57%)	0.003
Hispanic ethnicity		119 (10%)	103 (11%)	16 (10%)	1.0
Body Mass Index, Kg/m^2		28.3 (5.7)	28.2 (5.7)	29.0 (5.6)	0.09
Cause of ESKD	**Diabetes**	358 (32%)	315 (32%)	43 (27%)	0.1
	Hypertension	319 (28%)	271 (28%)	48 (30%)	
	Glomerulonephritis	183 (16%)	156 (16%)	27 (17%)	
	Graft failure	91 (8%)	71 (7%)	20 (13%)	
	Other	185 (16%)	164 (17%)	21 (13%)	
Preemptive transplant		117 (10%)	105 (11%)	12 (8%)	0.2
Previous kidney transplant		161 (14%)	130 (13%)	31 (19%)	0.04
Panel Reactive Antibody (PRA)	**0%**	729 (64%)	640 (66%)	89 (56%)	0.05
	1-20%	86 (8%)	70 (7%)	16 (10%)	
	21-80%	147 (13%)	127 (13%)	20 (13%)	
	>80%	174 (15%)	140 (14%)	34 (21%)	
HLA mismatch level		4.36 (1.33)	4.32 (1.34)	4.57 (1.23)	0.02
Induction Immunosuppression	**Anti-thymocyte globulin**	937 (82%)	803 (82%)	134 (85%)	0.5
	Basiliximab	167 (15%)	148 (15%)	19 (12%)	0.4
	Alemtuzumab	35 (3%)	30 (3%)	5 (3%)	0.7
	Rituximab	16 (1%)	12 (1%)	4 (3%)	0.3
Maintenance Immunosuppression At Discharge	**Prednisone**	945 (84%)	816 (85%)	129 (82%)	0.7
	Tacrolimus	1087 (96%)	947 (97%)	140 (89%)	<.001
	Cyclosporine	17 (1%)	11 (1%)	6 (4%)	0.04
	Mycophenolate	1098 (97%)	951 (97%)	147 (93%)	0.02
Delayed Graft Function		426 (37%)	340 (35%)	86 (54%)	<.001

* Non-event means that recipients did not experience the composite events of acute rejection or graft failure, but may have died. 36 deaths were included in the non-event group.
** Composite event includes acute rejection or graft failure not from death within 1 year.
Results are presented as mean (SD) or n (%).
ESKD, end-stage kidney disease; HLA, human leukocyte antigen.
Induction immunosuppression was missing in <3% of recipients, and discharge immunosuppression was missing in <1% of recipients.

Table 1b: Allograft and donor characteristics, by primary outcome status

Donor Characteristics		All (N=1137)*	Non-event (including death)** (N=978)	Composite event*** (N=159)	P
Mean age (Years)		41.5 (15.3)	41.0 (15.4)	44.7 (14.1)	0.005
Male		700 (62%)	609 (62%)	91 (57%)	0.2
Black race		183 (16%)	147 (15%)	36 (23%)	0.02
Hispanic ethnicity		167 (15%)	148 (15%)	19 (12%)	0.3
Body Mass Index, Kg/m^2		28.3 (7.4)	28.2 (7.1)	29.0 (9.5)	0.9
Hypertension		353 (31%)	293 (30%)	60 (38%)	0.05
Diabetes		118 (10%)	94 (10%)	24 (15%)	0.04
Cause of Death	Head Trauma	304 (27%)	258 (27%)	46 (29%)	0.4
	Anoxia	410 (37%)	362 (38%)	48 (31%)	
	Stroke	389 (35%)	330 (34%)	59 (38%)	
	Other	16 (1%)	13 (1%)	3 (2%)	
Hepatitis C seropositive		30 (3%)	27 (3%)	3 (2%)	0.5
DCD		217 (19%)	192 (20%)	25 (16%)	0.2
KDRI		1.31 (0.43)	1.3 (0.43)	1.41 (0.42)	<.001
KDPI, %		49.6 (27.3)	48.5 (27.3)	57.0 (26.0)	<.001
KDPI > 85%		126 (11%)	105 (9%)	21 (2%)	0.2
ECD		236 (21%)	197 (20%)	39 (25%)	0.2
Admission Creatinine (mg/dL)		1.11 (0.63)	1.12 (0.6)	1.07 (0.75)	0.02
Terminal Serum Creatinine (mg/dL)		1.21 (0.93)	1.2 (0.92)	1.24 (0.98)	0.6
Donor Cerebrovascular/stroke as cause of death	No	735 (65%)	638 (65%)	97 (61%)	0.3
	Yes	401 (35%)	339 (35%)	62 (39%)	
Donor AKI Stage	No AKI	827 (73%)	717 (74%)	110 (70%)	0.2
	Stage 1	184 (16%)	156 (16%)	28 (18%)	
	Stage 2	69 (6%)	61 (6%)	8 (5%)	
	Stage 3	51 (5%)	39 (4%)	12 (8%)	
Number of individual kidneys transplanted	1	86 (8%)	70 (7%)	16 (10%)	0.2
	2	1050 (92%)	907 (93%)	143 (90%)	
Kidney biopsied		591 (52%)	500 (51%)	91 (57%)	0.2
Kidney pumped		541 (48%)	465 (48%)	76 (48%)	1.0
Cold ischemia time (hours)		16.34 (6.98)	16.34 (7)	16.31 (6.89)	1.0

* 1137 kidneys were procured from 862 total donors.
** Non-event means that recipients did not experience the composite events of acute rejection or graft failure, but may have died. 36 deaths were included in the non-event group.
*** Composite event includes acute rejection or graft failure not from death within 1 year.
Results are presented as means (SD) or n (%).
ECD, expanded-criteria donor; DCD, donation after cardiovascular determination of death, KDRI Kidney donor risk index, KDPI kidney donor profile index
BMI and KDRI was missing in 5 donors. Admission Serum Creatinine was missing in 6 donors.

Table 2: Multivariable analysis of donor urinary biomarkers and primary composite outcome, using Fine-Gray competing risks models

Biomarker		Biomarker Range | n	Subdistribution Hazard Ratio (95% CI)			
			Unadjusted Model	Adjusted for KDRI	Model Adjusted for KDRI and Clinical Covariates*	Model Adjusted for KDRI, Urine Creatinine and Clinical Covariates*
IL-18	Log2-Trans	(1.367, 10.501) | n=1105	1.00 (0.91, 1.1)	1.00 (0.91, 1.11)	1.00 (0.9, 1.11)	0.98 (0.88, 1.09)
	Lower Tertile	(2.58, 28.24) | n=368	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Mid Tertile	(28.32, 78.88) | n=369	1.12 (0.73, 1.72)	1.19 (0.77, 1.83)	1.19 (0.77, 1.84)	1.1 (0.7, 1.73)
	Upper Tertile	(78.9, 1448.69) | n=368	0.85 (0.54, 1.34)	0.88 (0.55, 1.4)	0.86 (0.52, 1.41)	0.76 (0.45, 1.28)
KIM-1	Log2-Trans	(5.882, 15.205) | n=1105	1.04 (0.94, 1.16)	1.05 (0.95, 1.17)	1.06 (0.95, 1.18)	1.04 (0.91, 1.18)
	Lower Tertile	(58.96, 890.91) | n=369	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Mid Tertile	(894.16, 2503.7) | n=368	1.23 (0.79, 1.93)	1.33 (0.84, 2.1)	1.37 (0.86, 2.2)	1.33 (0.83, 2.15)
	Upper Tertile	(2521.91, 37759.01) | n=368	1.22 (0.78, 1.9)	1.29 (0.82, 2.03)	1.3 (0.8, 2.11)	1.2 (0.69, 2.07)
NGAL	Log2-Trans	(-3.322, 13.102) | n=1094	1.03 (0.96, 1.09)	1.03 (0.97, 1.1)	1.04 (0.97, 1.11)	1.03 (0.96, 1.11)
	Lower Tertile	(0, 20.6) | n=368	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Mid Tertile	(20.7, 104.6) | n=369	1.04 (0.66, 1.62)	1.05 (0.67, 1.66)	1.04 (0.65, 1.66)	1 (0.62, 1.61)
	Upper Tertile	(105, 8792.38) | N=368	1.12 (0.72, 1.73)	1.13 (0.72, 1.77)	1.19 (0.75, 1.89)	1.14 (0.71, 1.84)

* Clinical Covariates include cold ischemia time and the following recipient variables: age (years), black race, sex, previous kidney transplant, cause of end-stage kidney disease (4 categories, other as reference), number of human leukocyte antigen mismatches, panel reactive antibody (%), body mass index (kg/m^2), and pre-emptive transplant

Table 3: Multivariable analysis of donor urinary biomarkers and the secondary outcome of graft failure, acute rejection, and/or de novo donor specific antibody, using Fine-Gray competing risks models

Biomarker		Biomarker Range | n	Subdistribution Hazard Ratio (95% CI)			
			Unadjusted Model	Adjusted for KDRI	Model Adjusted for KDRI and Clinical Covariates*	Model Adjusted for KDRI, Urine Creatinine and Clinical Covariates*
IL-18	Log2-Trans	(1.367, 10.501) | n=409	**0.89 (0.79, 1)**	0.89 (0.79, 1)	0.91 (0.8, 1.04)	0.9 (0.78, 1.05)
	Lower Tertile	(2.58, 28.05) | n=143	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Mid Tertile	(28.32, 78.88) | n=122	0.94 (0.57, 1.58)	0.94 (0.56, 1.57)	0.98 (0.56, 1.71)	0.99 (0.5, 1.94)
	Upper Tertile	(78.9, 1448.69) | n=144	0.76 (0.45, 1.28)	0.77 (0.45, 1.3)	0.88 (0.48, 1.63)	0.81 (0.42, 1.56)
KIM-1	Log2-Trans	(5.882, 15.205) | n=409	**0.88 (0.78, 0.99)**	0.89 (0.78, 1.01)	0.91 (0.78, 1.05)	0.92 (0.77, 1.1)
	Lower Tertile	(58.96, 874.35) | n=137	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Mid Tertile	(899.99, 2503.7) | n=134	0.73 (0.44, 1.22)	0.83 (0.49, 1.38)	0.73 (0.41, 1.3)	0.88 (0.46, 1.69)
	Upper Tertile	(2521.91, 37759.01) | n=138	0.7 (0.42, 1.17)	0.74 (0.44, 1.24)	0.74 (0.41, 1.36)	0.9 (0.43, 1.87)
NGAL	Log2-Trans	(-3.322, 13.102) | n=406	**0.9 (0.84, 0.97)**	**0.92 (0.85, 0.99)**	0.93 (0.85, 1.02)	0.92 (0.83, 1.01)
	Lower Tertile	(0, 20.2) | n=158	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Mid Tertile	(20.8, 104.3) | n=133	0.73 (0.45, 1.19)	0.77 (0.47, 1.27)	0.79 (0.44, 1.41)	0.63 (0.33, 1.23)
	Upper Tertile	(105.1, 8792.38) | n=118	0.62 (0.36, 1.09)	0.71 (0.4, 1.25)	0.77 (0.42, 1.41)	0.66 0.34, 1.29)

* Clinical Covariates include cold ischemia time and the following recipient variables: age (years), black race, sex, previous kidney transplant, cause of end-stage kidney disease (4 categories, other as reference), number of human leukocyte antigen mismatches, panel reactive antibody (%), body mass index (kg/m^2), and pre-emptive transplant