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

Peter P. Reese, Mona D. Doshi, Isaac E. Hall, Behdad Besharatian, Jonathan S. Bromberg, Heather Thiessen-Philbrook, Yaqi Jia, Malek Kamoun, Sherry G. Mansour, Enver Akalin, Meera N. Harhay, Sumit Mohan, Thangamani Muthukumar, Bernd Schröppel, Pooja Singh, Francis L. Weng, and Chirag R. Parikh

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 that 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 1,137 kidney recipients at 13 centers.

Exposures: We measured concentrations of interleukin 18 (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 (AKIN) 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 posttransplant year.

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

Results: Mean recipient age was 54 ± 13 (SD) years, and 82% received antithymocyte globulin. We found no significant associations between donor urinary IL-18, KIM-1, and NGAL and the primary outcome (subdistribution hazard ratio [HR] for highest vs lowest tertile of 0.76 [95% CI, 0.45-1.28], 1.20 [95% CI, 0.69-2.07], and 1.14 [95% CI, 0.71-1.84], respectively). In secondary analyses, we detected no significant associations between clinically defined AKI and the primary outcome or between donor biomarkers and the composite outcome of BPAR, graft failure, and/or de novo DSA.

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

Conclusions: In a large cohort of kidney recipients who almost all received induction with thymoglobulin, donor injury biomarkers were associated with neither graft failure and rejection nor 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.

Visual Abstract online

Complete author and article information provided before references.

Correspondence to C.R. Parikh (chirag.parikh@jhmi.edu)

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Kidneys from deceased organ donors often are affected by acute kidney injury (AKI) 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 in 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 (eg, the mannose-binding lectin pathway) and enhanced expression of toll-like receptors (TLRs) 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 that the recipients of AKI kidneys would experience increased rates of acute rejection, both cellular and antibody, and formation of de novo donor-specific antibody (DSA).

The "injury hypothesis" was proposed more than 20 years ago (with subsequent modifications) and states that oxidative stress and injury to the kidney at procurement and transplant variably activate innate immunity in the allograft, which affects alloimmune responses and increases the risk of rejection.[5] Classically, this injury pathway would be expected to provoke acute cellular rejection via alloreactive T cells. However, B-cell responses may be affected concurrently and promote acute antibody-mediated rejection via DSA. Antibody-mediated rejection may carry a worse prognosis than acute cellular rejection, 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 multicenter, prospective cohort (the Deceased Donor Study) that included testing deceased-donor urine for injury biomarkers and detailed chart review of recipient outcomes, including biopsies. We identified AKI using conventional serological definitions that rely on changes in serum creatinine (Scr) concentration as well as characterizing injury using sensitive urinary biomarkers including interleukin 18 (IL-18), kidney injury molecule 1 (KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL). We showed that AKI defined using Scr (corresponding to stage

PLAIN-LANGUAGE SUMMARY

Many patients in the United States wait years for a 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 transplant. We measured the level of kidney injury in the urine of 862 donors. We then studied the clinical outcomes for the 1,137 adult recipients of kidneys transplanted from those donors. Compared with 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.

2 or worse by Acute Kidney Injury Network [AKIN] criteria) was present in approximately 9% of deceased-donor kidneys and that many additional donors had increased 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 those for 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 transplant.[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 posttransplant 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, 5 participating organ procurement organizations (OPOs) enrolled donors between May 2010 and December 2013. Each OPO used 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 at 13 participating transplant centers who were at least 16 years of age and 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). This data system includes data on all donors, waitlisted candidates, and transplant recipients in the United States as submitted by OPTN members, and has been described elsewhere. The Health Resources and Services Administration, which is part of the US 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 the requirement for 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 BPAR in the first posttransplant 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 5 of the participating centers that screened recipients for de novo DSA; we did not include centers that measured DSA only for clinical concern for rejection.

For the binary outcome of de novo DSA, each center applied the criterion of mean fluorescence intensity (MFI) of at least 1,000. For patients with pretransplant DSA, we also categorized as a positive outcome an MFI of at least 1,000 and ≥50% higher than the pretransplant DSA MFI. MFI was defined as antibody reactivity to the specific donor HLA allele in cases in which allele level typing was available. When 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 1 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 immunosuppression nonadherence.

Donor Urine Injury Biomarker Data

The primary exposure was donor urinary concentrations of IL-18 (in pg/mL), KIM-1 (in pg/mL), and NGAL (in ng/dL). Before 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 °C until the next monthly shipment to the central study biorepository. Biomarker measurements are described thoroughly in Item S3 and in previous work.[10]

Statistical Analysis

Item S4 includes details about the calculation of variables. We calculated descriptive statistics as means ± SD, medians (IQR), or frequencies (percentages). Donor, transplant, and recipient characteristics were compared by primary outcome using Kruskal-Wallis or χ^2 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 HR for donor injury biomarkers and the primary outcome, with death as a competing risk. Donor injury biomarkers were both modeled continuously after a $\log_2$ 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 2 recipients using robust sandwich estimates. In the primary analysis, we adjusted for variables available at organ offer and collected by OPOs: the KDRI (Kidney Donor Risk Index), the transplant variables cold ischemia time (in hours) and number of HLA mismatches, and the following recipient variables: age (years), sex, Black race, previous kidney transplant, cause of kidney failure, percent panel reactive antibody (PRA), body mass index, and preemptive transplant.[10,19] In the analysis of the secondary outcome (that included de novo DSA), we also adjusted for pretransplant 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 (Kidney Donor Profile Index; cutoff, 85%), donation after circulatory determination of death (DCD), kidney machine perfusion, cold ischemia time (median value cutoff, 14 hours), DGF, donor-recipient sex combinations,[17,20] and 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 (1) donor clinical AKI and the outcome of BPAR only and (2) 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 α of 5%, power of 80%, that the HR 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 an HR of at least 0.56 (within the cohort of 1,137 recipients). For the secondary outcome of BPAR, graft failure, or DSA, we estimated the power to detect an HR of at least 0.47 (within the cohort of 422 recipients).

We used SAS 9.4 for Windows (SAS Institute). All statistical tests and confidence intervals were 2-sided with a significance level of 0.05.

Results

A shown in Fig 1, the primary cohort comprised 1,137 deceased-donor kidney transplant recipients at 13 centers. Table 1 shows that the mean recipient age was 53.7 ± 13.3 (SD) years and 61% were male. Fourteen percent had previously received kidney transplants, and 15% had estimated PRA >80%. Eighty-two percent of recipients received rabbit antithymocyte globulin induction therapy, 15% received basiliximab, and 3% received alemtuzumab. Table S1 provides additional details about immunosuppression. Compared with 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 (57% vs 45%; $P = 0.003$), to have prior transplants (19% vs 13%; $P = 0.04$), and, in a finding of borderline statistical significance, to have calculated PRA titers >80% (21% vs 14%; $P = 0.05$ for the association with all 4 levels of PRA); however, they were less likely to be discharged from the transplant hospitalization on tacrolimus (89% vs 97%; $P < 0.001$) or mycophenolate (93% vs 97%; $P = 0.02$). A total of 37% of recipients experienced DGF. Recipients who experienced the primary outcome were also more likely to have DGF (54% vs 35% for those without the primary outcome; $P < 0.001$).

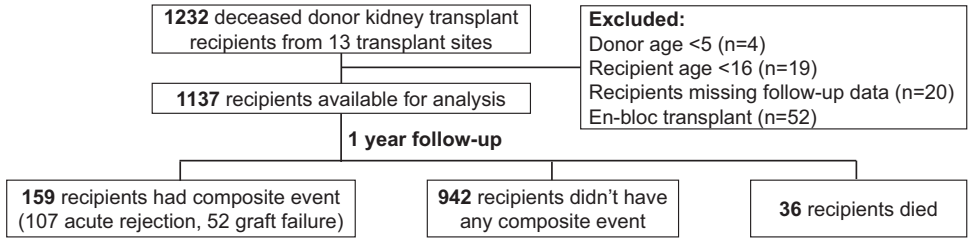

Figure 1. Flow chart for the primary cohort.

For the deceased kidney donors, mean terminal Scr concentration was 1.21 ± 0.93 mg/dL, and 19% were DCDs (Table 1). 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 (107 met the primary outcome because of BPAR). Table S2 shows time-to-event data. A total of 77 of BPAR episodes were acute cellular rejection only, 8 were antibody-mediated rejection only, 12 were both acute cellular rejection and antibody-mediated rejection, 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 comparing highest- versus lowest-tertile biomarker concentrations, the subdistribution HRs were 0.76 (95% CI, 0.45-1.28) for IL-18, 1.20 (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 constituted the subcohort with DSA screening (Fig S1). Fifty-four (13%) had pretransplant DSA. By 1 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 1 year. We found no significant association between urinary injury biomarkers and the secondary composite outcome. In the fully adjusted models comparing highest- versus lowest-tertile biomarker concentrations, the subdistribution HRs 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 and 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 and S8, donor urinary biomarkers were significantly associated with neither the outcome of BPAR nor 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-S12).

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 transplant. 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 because of concerns about early clinical complications such as primary nonfunction and longer-term risks of allograft fibrosis.[1] Yu et al examined kidney nonprocurement among deceased donors in the United States in 2000-2018. Compared with donors with terminal Scr <1.00 mg/dL, those with values between 1.00 and 1.49 mg/dL and between 1.50 and 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 Scr >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 kidneys and 15.1% for

Table 1. Characteristics of Recipients as Well as Donors and Allografts by Primary Outcome Status

	All (N = 1,137)[a]	Non-Event (Including Death)[b] (n = 978)	Composite Event[c] (n = 159)	*P*
Recipient Characteristics				
Age, y	53.7 ± 13.3	53.7 ± 13.3	53.7 ± 13.4	0.9
Male sex	693 (61%)	597 (61%)	96 (60%)	0.9
Black race	528 (46%)	437 (45%)	91 (57%)	0.003[d]
Hispanic ethnicity	119 (10%)	103 (11%)	16 (10%)	0.9
Body mass index, kg/m^2	28.3 ± 5.7	28.2 ± 5.7	29.0 ± 5.6	0.09[d]
Cause of kidney failure				0.1
Diabetes	358 (32%)	315 (32%)	43 (27%)	
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[d]
PRA				0.05[d]
0%	729 (64%)	640 (66%)	89 (56%)	
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[d]
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	1,087 (96%)	947 (97%)	140 (89%)	<0.001[d]
Cyclosporine	17 (1%)	11 (1%)	6 (4%)	0.04[d]
Mycophenolate	1,098 (97%)	951 (97%)	147 (93%)	0.02[d]
Delayed graft function	426 (37%)	340 (35%)	86 (54%)	<0.001[d]
Allograft and Donor Characteristics				
Age, y	41.5 ± 15.3	41.0 ± 15.4	44.7 ± 14.1	0.005[d]
Male sex	700 (62%)	609 (62%)	91 (57%)	0.2
Black race	183 (16%)	147 (15%)	36 (23%)	0.02[d]
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[d]
Diabetes	118 (10%)	94 (10%)	24 (15%)	0.04[d]
Cause of death				0.4
Head trauma	304 (27%)	258 (27%)	46 (29%)	
Anoxia	410 (37%)	362 (38%)	48 (31%)	
Stroke	389 (35%)	330 (34%)	59 (38%)	
Other	16 (1%)	13 (1%)	3 (2%)	
HCV 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	<0.001[d]
KDPI, %	49.6 ± 27.3	48.5 ± 27.3	57.0 ± 26.0	<0.001[d]
KDPI >85%	126 (11%)	105 (9%)	21 (2%)	0.2
ECD	236 (21%)	197 (20%)	39 (25%)	0.2
Admission Scr, mg/dL	1.11 ± 0.63	1.12 ± 0.6	1.07 ± 0.75	0.02[d]
Terminal Scr, mg/dL	1.21 ± 0.93	1.2 ± 0.92	1.24 ± 0.98	0.6
Donor CBVD/stroke as cause of death				0.3
No	735 (65%)	638 (65%)	97 (61%)	
Yes	401 (35%)	339 (35%)	62 (39%)	

(Continued)

Table 1 (Cont'd). Characteristics of Recipients as Well as Donors and Allografts by Primary Outcome Status

	All (N = 1,137)[a]	Non-Event (Including Death)[b] (n = 978)	Composite Event[c] (n = 159)	*P*
Donor AKI stage				0.2
No AKI	827 (73%)	717 (74%)	110 (70%)	
Stage 1	184 (16%)	156 (16%)	28 (18%)	
Stage 2	69 (6%)	61 (6%)	8 (5%)	
Stage 3	51 (5%)	39 (4%)	12 (8%)	
No. of individual kidneys transplanted				0.2
1	86 (8%)	70 (7%)	16 (10%)	
2	1,050 (92%)	907 (93%)	143 (90%)	
Kidney biopsied	591 (52%)	500 (51%)	91 (57%)	0.2
Kidney pumped	541 (48%)	465 (48%)	76 (48%)	0.9
Cold ischemia time, h	16.34 ± 6.98	16.34 ± 7.00	16.31 ± 6.89	0.9

Results are presented as mean ± SD or n (%). Induction immunosuppression was missing in <3% of recipients, and discharge immunosuppression was missing in <1% of recipients. Body mass index and KDRI were missing in 5 donors; admission Scr in 6 donors. Abbreviations: CBVD, cerebrovascular disease; DCD, donation after cardiovascular determination of death; ECD, expanded-criteria donor; KDPI, Kidney Donor Profile Index; KDRI, Kidney Donor Risk Index; PRA, panel reactive antibody; Scr, serum creatinine.
[a]A total of 1,137 kidneys were procured from 862 total donors.
[b]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.
[c]Composite event includes acute rejection or graft failure not from death within 1 year.
[d]Significant at $P \leq 0.05$.

recipients of non-AKI kidneys. In multivariable adjustment, AKI kidneys were associated with only slightly higher risk of all-cause graft failure (adjusted HR, 1.05 [95% CI, 1.01-1.09]).[9] Prior studies from our Deceased Donor Study cohort examined AKI using Scr-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 level was associated with recipient DGF (relative risk for highest vs lowest NGAL tertile, 1.21 [95% CI, 1.02-1.43]). However, analyses of 6-month recipient estimated glomerular filtration rate revealed that NGAL and liver fatty acid binding protein were associated with only modestly lower estimated glomerular filtration rate, and this association was restricted to recipients without DGF.[10] A study from the United Kingdom reported higher primary

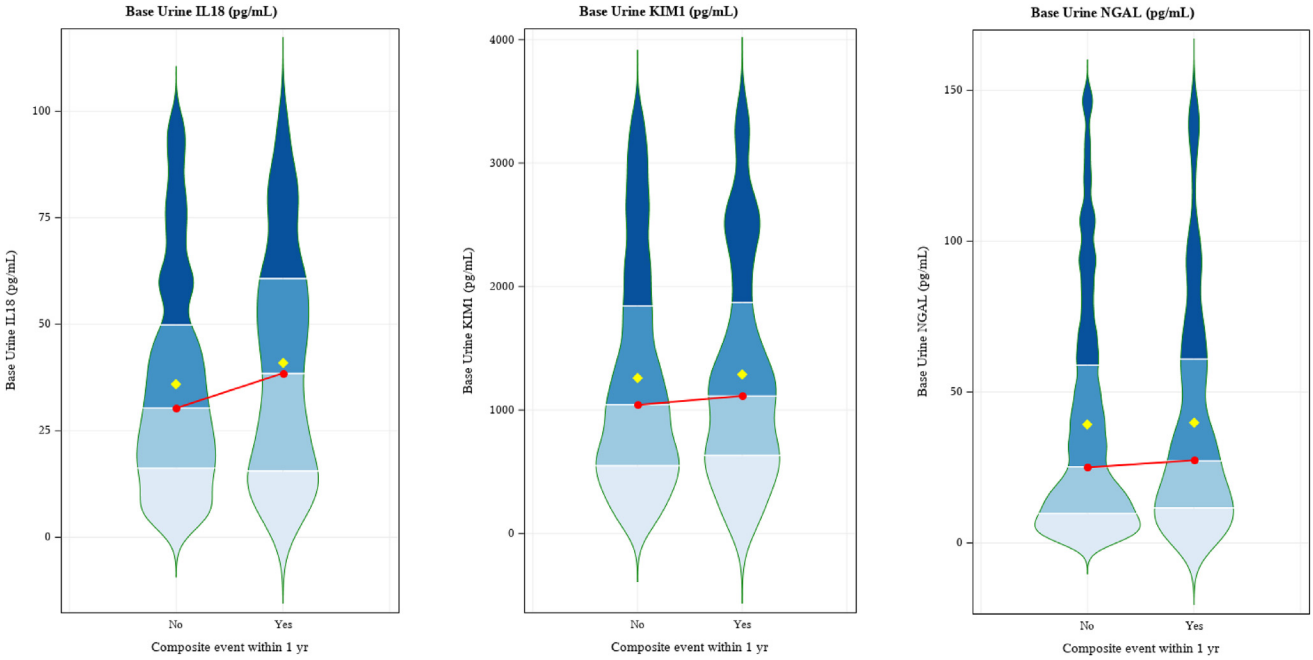

Figure 2. Donor urinary biomarker distributions among recipients who did and did not experience the primary composite outcome of graft failure or acute rejection. Abbreviations: IL-18, interleukin 18; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin.

Table 2. Multivariable Analysis of Donor Urinary Biomarkers and Primary Composite Outcome Using Fine-Gray Competing Risks Models

		sHR (95% CI) for Model			
Biomarker	Biomarker Range; n	Unadjusted	Adj for KDRI	Adj for KDRI + Clinical Covariates[a]	Adj for KDRI, Urine Creatinine, Clinical Covariates[a]
IL-18					
Continuous[b]	1.367 to 10.501; n = 1,105	1.00 (0.91-1.10)	1.00 (0.91-1.11)	1.00 (0.90-1.11)	0.98 (0.88-1.09)
Lower tertile	2.58 to 28.24; n = 368	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	28.32 to 78.88; n = 369	1.12 (0.73-1.72)	1.19 (0.77-1.83)	1.19 (0.77-1.84)	1.1 (0.70-1.73)
Upper tertile	78.9 to 1,448.69; n = 368	0.85 (0.54-1.34)	0.88 (0.55-1.40)	0.86 (0.52-1.41)	0.76 (0.45-1.28)
KIM-1					
Continuous[b]	5.882 to 15.205; n = 1,105	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 to 890.91; n = 369	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	894.16 to 2,503.7; n = 368	1.23 (0.79-1.93)	1.33 (0.84-2.10)	1.37 (0.86-2.20)	1.33 (0.83-2.15)
Upper tertile	2,521.91 to 37,759.01; n = 368	1.22 (0.78-1.90)	1.29 (0.82-2.03)	1.3 (0.80-2.11)	1.2 (0.69-2.07)
NGAL					
Continuous[b]	−3.322 to 13.102; n = 1,094	1.03 (0.96-1.09)	1.03 (0.97-1.10)	1.04 (0.97-1.11)	1.03 (0.96-1.11)
Lower tertile	0 to 20.6; n = 368	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	20.7 to 104.6; n = 369	1.04 (0.66-1.62)	1.05 (0.67-1.66)	1.04 (0.65-1.66)	1.00 (0.62-1.61)
Upper tertile	105 to 8,792.38; 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)

Abbreviations: Adj, adjusted; HR, hazard ratio; KDRI, Kidney Donor Risk Index; NGAL, neutrophil gelatinase-associated lipocalin.
[a]Clinical covariates include cold ischemia time and the following recipient variables: age (years), Black race, sex, previous kidney transplant, cause of kidney failure (4 categories, other as reference), number of HLA mismatches, panel reactive antibody (%), body mass index (kg/m^2), and preemptive transplant.
[b]Values $\log_2$-transformed. sHR is per 1-unit greater value.

nonfunction rates for stage 3 AKI kidneys (9% vs 4%; P = 0.04) and advised caution about accepting these kidneys,[2] but those results contrast with findings from multiple other single-center and multicenter studies that have described favorable outcomes after kidney transplant 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 the following 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

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

		Subdistribution Hazard Ratio (95% CI) for Model			
Biomarker	Biomarker Range; n	Unadjusted	Adj for KDRI	Adj for KDRI + Clinical Covariates[a]	Adj for KDRI, Urine Creatinine, Clinical Covariates[a]
IL-18					
Continuous[b]	1.367 to 10.501; n = 409	0.89 (0.79-1.00)	0.89 (0.79-1.00)	0.91 (0.80-1.04)	0.90 (0.78-1.05)
Lower tertile	2.58 to 28.05; n = 143	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	28.32 to 78.88; n = 122	0.94 (0.57-1.58)	0.94 (0.56-1.57)	0.98 (0.56-1.71)	0.99 (0.50-1.94)
Upper tertile	78.9 to 1,448.69; n = 144	0.76 (0.45-1.28)	0.77 (0.45-1.30)	0.88 (0.48-1.63)	0.81 (0.42-1.56)
KIM-1					
Continuous[b]	5.882 to 15.205; n = 409	0.88 (0.78-0.99)	0.89 (0.78-1.01)	0.91 (0.78-1.05)	0.92 (0.77-1.10)
Lower tertile	58.96 to 874.35; n = 137	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	899.99 to 2,503.7; n = 134	0.73 (0.44-1.22)	0.83 (0.49-1.38)	0.73 (0.41-1.30)	0.88 (0.46-1.69)
Upper tertile	2,521.91 to 37,759.01; 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					
Continuous[b]	−3.322 to 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 to 20.2; n = 158	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Middle tertile	20.8 to 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 to 8,792.38; n = 118	0.62 (0.36-1.09)	0.71 (0.40-1.25)	0.77 (0.42-1.41)	0.66 (0.34-1.29)

Abbreviations: Adj, adjusted; HR, hazard ratio; KDRI, Kidney Donor Risk Index; NGAL, neutrophil gelatinase-associated lipocalin.
[a]Clinical covariates include cold ischemia time and the following recipient variables: age (years), Black race, sex, previous kidney transplant, cause of kidney failure (4 categories, other as reference), number of HLA mismatches, panel reactive antibody (%), body mass index (kg/m^2), and preemptive transplant.
[b]Values $\log_2$-transformed. sHR is per 1-unit greater value.

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 increased injury markers actually experienced better 6-month graft function than 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 injury.[10] Next, we note that 82% of recipients received antithymocyte 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 contrary, our findings suggest that, even if AKI caused subclinical rejection, the clinical consequences were limited, perhaps because of 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, perhaps one 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 still-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 5 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 and 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 multicenter study with close follow-up of recipients, donor injury biomarkers were associated with neither the primary outcome of graft failure and rejection nor 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.

Supplementary Material

Supplementary File (PDF)

Figure S1: Flow chart for the 5-center subcohort.

Item S1: Supplementary methods and data quality assurance.

Item S2: Center protocols for screening for DSA.

Item S3: Methods related to measurement of urinary biomarkers.

Item S4: Variables.

Table S1: Transplant recipients' immunosuppression medications and other exposures, by composite event.

Table S2: Distribution of the number of days between transplant and study outcomes.

Table S3: Classification of rejection outcomes.

Table S4: DSA details for 35 recipients with de novo DSA in the 5-center subcohort.

Table S5: Exploratory analyses of effect modification between donor urinary biomarkers, prespecified clinical characteristics, and the primary outcome.

Table S6: Unadjusted HRs for NGAL and the primary outcome, by DCD status.

Table S7: Multivariable analysis of donor urine biomarkers and the BPAR outcome, using Fine-Gray competing risk models.

Table S8: Multivariable analysis of donor urinary biomarkers and composite outcome of BPAR, graft failure, or death.

Table S9: Multivariable analysis of clinically defined donor AKI and primary composite outcome of graft failure or BPAR, using Fine-Gray competing risks models.

Table S10: Multivariable analysis of clinically defined donor AKI and the secondary composite outcome of graft failure, BPAR, or de novo DSA, using Fine-Gray competing risks models.

Table S11: Multivariable analysis of clinically defined donor AKI and the exploratory outcome of BPAR, using Fine-Gray competing risks models.

Table S12: Multivariable analysis of clinically defined donor AKI and the exploratory composite outcome of BPAR, graft failure, or death.

Article Information

Authors' Full Names and Academic Degrees: Peter P. Reese, MD, PhD, Mona D. Doshi, MD, Isaac E. Hall, MD, MS, Behdad Besharatian, MD, Jonathan S. Bromberg, MD, PhD, Heather Thiessen-Philbrook, MMath, Yaqi Jia, MPH, Malek Kamoun, MD, PhD, Sherry G. Mansour, DO, MS, Enver Akalin, MD, Meera N. Harhay, MD, MSCE, Sumit Mohan, MD, MPH, Thangamani Muthukumar, MD, Bernd Schröppel, MD, Pooja Singh, MD, Francis L. Weng, MD, MSCE, and Chirag R. Parikh, MD, PhD.

Authors' Affiliations: Department of Biostatistics, Epidemiology and Informatics (PPR), Renal-Electrolyte and Hypertension Division, Department of Medicine (PPR, BB), and Department of Pathology and Laboratory Medicine (MK), Perelman School of Medicine,

University of Pennsylvania; Department of Medicine, Drexel University College of Medicine (MNH), Department of Epidemiology and Biostatistics, Drexel University Dornsife School of Public Health (MNH), Department of Medicine, Division of Nephrology, Sidney Kimmel Medical College, Thomas Jefferson University Hospital (PS), Philadelphia, PA; Division of Nephrology, Department of Medicine, University of Michigan, Ann Arbor, MI (MDD); Division of Nephrology and Hypertension, Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT (IEH); Department of Surgery, Division of Transplantation and Department of Microbiology and Immunology, University of Maryland School of Medicine (JSB), Division of Nephrology, Department of Medicine, Johns Hopkins University School of Medicine (HT-P, YJ, CRP), Baltimore, MD; Program of Applied Translational Research and Department of Internal Medicine, Section of Nephrology, Yale University School of Medicine, New Haven, CT (SGM); Kidney Transplant Program, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx (EA), Department of Epidemiology, Columbia University Mailman School of Public Health (SM), Department of Medicine, Division of Nephrology, Columbia University Vagelos College of Physicians and Surgeons (SM), Department of Medicine, Division of Nephrology and Hypertension and Department of Transplantation Medicine (TM), New York Presbyterian Hospital-Weill Cornell Medical Center, New York, NY; Section of Nephrology, University Hospital, Ulm, Germany (BS); and Renal and Pancreas Transplant Division at Cooperman Barnabas Medical Center, RWJ Barnabas Health, Livingston, NJ (FLW).

Address for Correspondence: Chirag R. Parikh, MD, PhD, Division of Nephrology, Department of Medicine, Johns Hopkins University School of Medicine, 1830 E Monument St, Suite 416, Baltimore, MD 21287. Email: chirag.parikh@jhmi.edu

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: HT-P, 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.

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