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Associations Between Deceased-Donor Urine MCP-1 and Kidney Transplant Outcomes

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Introduction: Existing methods to predict recipient allograft function during deceased-donor kidney procurement are imprecise. Understanding the potential renal reparative role for monocyte chemoattractant protein-1 (MCP-1), a cytokine involved in macrophage recruitment after injury, might help to predict allograft outcomes.

Methods: We conducted a substudy of the multicenter prospective Deceased Donor Study cohort that evaluated deceased kidney donors from 5 organ procurement organizations from May 2010 to December 2013. We measured urine MCP-1 (uMCP-1) concentrations from donor samples collected at nephrectomy to determine associations with donor acute kidney injury (AKI), recipient delayed graft function (DGF), 6-month estimated glomerular filtration rate (eGFR), and graft failure. We also assessed perfusate MCP-1 concentrations from pumped kidneys for associations with DGF and 6-month eGFR.

Results: AKI occurred in 111 donors (9%). The median (interquartile range) uMCP-1 concentration was higher in donors with AKI compared with donors without AKI (1.35 [0.41–3.93] ng/ml vs. 0.32 [0.11–0.80] ng/ml, $P < 0.001$). DGF occurred in 756 recipients (31%), but uMCP-1 was not independently associated with DGF. Higher donor uMCP-1 concentrations were independently associated with a higher 6-month eGFR in those without DGF (0.77 [0.10–1.45] ml/min per 1.73 m^2 per doubling of uMCP1). However, there were no independent associations between uMCP-1 and graft failure over a median follow-up of ~2 years. Lastly, perfusate MCP-1 concentrations significantly increased during pump perfusion but were not associated with DGF or 6-month eGFR.

Discussion: Donor uMCP-1 concentrations were modestly associated with higher recipient 6-month eGFR in those without DGF. However, the results suggest that donor uMCP-1 has minimal clinical utility given no associations with graft failure.

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In the United States, the growing number of patients on the kidney transplant waiting list increased by 50% between 2002 and 2013 to ~100,000 individuals.[1] Transplant rates have not met the increasing demand, with only 16% of wait-listed patients receiving a kidney transplant in 2013. To help address these shortcomings with better organ utilization, the National Kidney Allocation System was revised in December 2014 to incorporate the kidney donor profile index and restrict access to the highest quality kidneys, but there remains a compelling need for more reliable and accurate tools to assess donor kidney quality and graft outcomes.[2]

We evaluated several kidney injury biomarkers such as neutrophil gelatinase–associated lipocalin (NGAL), liver-type fatty acid binding protein, interleukin-18, and kidney injury molecule-1 in deceased-donor urine at the time of organ procurement, but only

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NGAL and liver-type fatty acid binding protein provided modest incremental value in predicting recipient kidney graft outcomes.[3] Other biological processes beyond structural kidney damage, such as inflammation and repair, are likely also activated at the time of organ procurement and affect recipient outcomes. Hence, we also evaluated a repair phase protein called Chitinase 3-like-1 (YKL-40) in deceased-donor urine and found that higher YKL-40 concentrations were associated with improved 6-month estimated glomerular filtration rate (eGFR) as well as a lower risk of graft failure.[4] This finding highlighted the pivotal role that renal recovery following ischemia-reperfusion injury plays in deceased-donor kidney transplantation. Thus, additional biomarkers of renal repair processes during organ procurement (especially in conjunction with injury biomarkers) might provide prognostic information regarding subsequent kidney allograft outcomes.

Increasing evidence indicates that monocyte chemoattractant protein-1 (MCP-1) is involved in the post-injury phase and is associated with inflammation, repair, and fibrosis in native kidney disease.[5,6] MCP-1, also called chemokine (CC-motif) ligand 2 (CCL2), is a cytokine that recruits inflammatory cells to sites of damage in response to tissue injury. It is mainly produced by monocytes and macrophages, but many other cells can express the protein in the setting of injury including epithelial cells, endothelial cells, and fibroblasts.[5] In the kidney transplant population, 6-month recipient urine MCP-1 (uMCP-1) concentrations correlated with interstitial fibrosis and inflammation in 24-month transplant biopsies, implicating the role of persistent MCP-1 in renal disease progression.[6] In addition, the so-called high MCP-1 producer genotype MCP-1-2518 (G/G) was also shown to be a risk factor for premature renal allograft failure.[7]

Although MCP-1 is generally regarded as a biomarker involved with the initiation of fibrosis, emerging evidence suggests that MCP-1 also plays an important role in repair after injury.[8,9] MCP-1–deficient mice demonstrated poor wound healing with reduced re-epithelialization and angiogenesis.[8] MCP-1–deficient mice also experienced more significant renal injury in the setting of ischemia-reperfusion, with increased proximal tubule apoptosis and worse survival compared with wild-type mice, indicating that renal MCP-1 expression is protective in the setting of ischemia-reperfusion injury.[9] Thus, MCP-1 appears to serve dual roles with tissue protection during the inflammatory phase of AKI to allow for successful renal repair or the development of fibrosis with prolonged expression during severe or ongoing injury. As MCP-1 is the main recruiting factor for macrophages, macrophages may also have a similar dual role in inflammation and repair. Preclinical studies have provided evidence of this in macrophage-depleted mice that were unable to initiate repair processes after renal ischemia-reperfusion injury or after unilateral ureteral obstruction.[10–12]

The involvement of MCP-1 in the repair and recovery of kidney function after injury remains to be fully investigated in humans. The potential for donor uMCP-1 concentrations to predict kidney transplant recipient outcomes has never been evaluated. We present a substudy of the multicenter, prospective Deceased Donor Study cohort study that assesses the associations of deceased-donor uMCP-1 concentrations at organ procurement as well as perfusate MCP-1 concentrations from kidneys on hypothermic machine perfusion (HMP) with recipient graft function.

METHODS

Study Design and Participants

We enrolled deceased organ donors in collaboration with 5 organ procurement organizations: 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. Donor variables were obtained from organ procurement organization donor charts, and recipient characteristics and outcomes were obtained from the Organ Procurement and Transplantation Network/United Network for Organ Sharing database. The Organ Procurement and Transplantation Network/United Network for Organ Sharing data system have been described in detail elsewhere.[13]

Briefly, deceased donors who were at least 16 years of age and whose family consented to research were included in the study. Donors with missing admission or terminal serum creatinine values or missing urine samples were not eligible for the study. We also excluded donor kidneys without associated MCP-1 measurements. The institutional review boards of all participating centers approved this study (Human Investigation Committee Protocol Number 1206010465).

Outcome Definitions

Donor AKI was defined by a greater than 2-fold increase in serum creatinine from admission to the terminal value irrespective of urine output or duration of time between the 2 measurements, which corresponded to stage 2 AKI by the Acute Kidney Injury Network criteria.[14] Recipient DGF was defined as requiring any dialysis in the first week post-transplantation. The 6-month eGFR was calculated by the Chronic

Kidney Disease Epidemiology Collaboration equation using the serum creatinine values reported to the United Network for Organ Sharing on 6-month follow-up forms.[15] Preference was given to 6-month creatinine values for graft function as opposed to later time points, as the reliability and quality of this variable from the United Network for Organ Sharing was validated by chart review.[16]

Sample Collection and uMCP-1 Measurement

Upon transfer to the donor operating room, 10 ml of urine was obtained from the catheter tubing and then transported on ice to the organ procurement organization, where it was stored at −80°C. Samples were delivered to the Yale University biorepository monthly. Upon arrival to the biorepository, samples underwent a single controlled thaw, were centrifuged at 5000*g* for 10 minutes at 4 °C, separated into 1-ml aliquots, and immediately stored at −80 °C until uMCP-1 measurement. uMCP-1 measurements were analyzed as concentrations (ng/ml) and indexed to urine creatinine (ng/mg) to account for dilution.

Hypothermic pulsatile flow via the LifePort Kidney Transporter was used for individually pumped kidneys. Perfusate measurements were obtained at 2 different time points. The initial sample (base) was taken within minutes of starting perfusion, and the second sample (post) was obtained before the Organ Procurement Organization transferred management of the kidney to the recipient center. Details regarding HMP and perfusate collection and storage were described in our previous publication.[17]

Urinary and perfusate MCP-1 were measured using 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 Analyses

All analyses were 2 tailed, and P values <0.05 were considered significant. Descriptive statistics for continuous variables were reported as mean (SD) or median (interquartile range) and as frequencies (%) for categorical variables. Donor, recipient, and clinical characteristics were analyzed using the Mann-Whitney Wilcoxon test for continuous variables and the χ^2 test for categorical variables. When evaluating the association between donor uMCP-1 concentrations and outcomes, donor uMCP-1 concentrations were analyzed both as continuous ($\log_2$-transformed uMCP-1) and categorical (uMCP-1 tertiles) variables. Outlier values for uMCP-1 concentrations were included in all analyses but were suppressed for visual clarity in graphic form. The associations between uMCP1 and the categorical outcomes of donor AKI, recipient DGF, and graft failure were analyzed using modified Poisson regression.[18] The association between uMCP-1 and the continuous outcome of the 6-month eGFR was analyzed using multivariable linear regression.

β Coefficients were estimated using the linear regression model, in which β was defined as the change in the 6-month eGFR associated with each $\log_2$-unit increase in uMCP-1, when all other variables were held fixed. Multivariable models for the outcome of donor AKI were adjusted for the following donor variables: age (years), height (cm), weight (kg), black race, history of hypertension, history of diabetes, stroke as the cause of death, donation after cardiac determination of death status. We excluded terminal serum creatinine when modeling for the outcome of donor AKI as this variable is used to define AKI itself. In addition to these donor variables, multivariable models for the outcomes of DGF, 6-month eGFR, and graft failure included adjustment for terminal serum creatinine (mg/dl), and urinary NGAL (uNGAL, ng/ml). The adjustment for uNGAL was performed to account for the severity of underlying ischemic tubular injury. We also adjusted for the following recipient and transport characteristics when assessing the outcomes of DGF, 6-month eGFR, and graft failure: age (years), black race, sex, previous kidney transplant, diabetes as the cause of end-stage renal disease, end-stage renal disease duration (months), number of human leukocyte antigen mismatches, body mass index (kg/m^2), panel reactive antibody (percentage), cold ischemia time (hours), and use of HMP. We performed a stratified analysis to determine whether the association between donor uMCP-1 concentrations and outcomes differed by donor AKI and recipient DGF status. The interaction between uMCP-1 and donor AKI as well as recipient DGF was tested in the unadjusted model. Full multivariable models including all covariates for the outcomes of DGF and 6-month eGFR are shown in Supplementary Tables S1 and S2.

If the recipient died before 6 months, we carried forward their last reported serum creatinine to calculate the eGFR. If the recipient experienced graft failure (return to dialysis or retransplantation) before 6 months, the 6-month eGFR was imputed as 10 ml/min per 1.73 m^2.

Analyses were performed using SAS 9.4 software for Windows (SAS Institute, Cary, NC) and R 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Of the 1679 donors enrolled, 1301 met inclusion criteria and were analyzed. A total of 2435 patients received

kidney transplants from these donors. The study cohort generation is shown in Figure 1. Donor characteristics stratified by AKI status are shown in Table 1. A total of 111 donors (9%) had AKI and 1190 (91%) did not have AKI. Donor characteristics were similar between groups except for admission and terminal creatinine, kidney donor risk index, kidney donor profile index, and number of kidneys transplanted.

uMCP-1 Concentrations and Donor AKI

As shown in Figure 2, median (interquartile range) uMCP-1 concentrations were significantly higher in donors with AKI compared with donors without AKI (1.35 [0.41–3.93] ng/ml vs. 0.32 [0.11–0.80] ng/ml, $P < 0.001$). A box plot with all outliers is also shown in Supplementary Figure S1, and histograms of the distribution of MCP-1 levels across the entire cohort and stratified by AKI status are shown in Supplementary Figures S2 and S3. As shown in Table 2, each $\log_2$-unit increase in uMCP-1 was associated with 54% increased risk of donor AKI after adjusting for donor characteristics (adjusted relative risk, 1.54; 95% confidence interval 1.42–1.67). A biological gradient was apparent between donor uMCP-1 concentrations and AKI. Compared with the lowest uMCP-1 tertile, the highest uMCP-1 tertile had an adjusted relative risk for donor AKI of 9.80 (95% confidence interval 4.75–20.23).

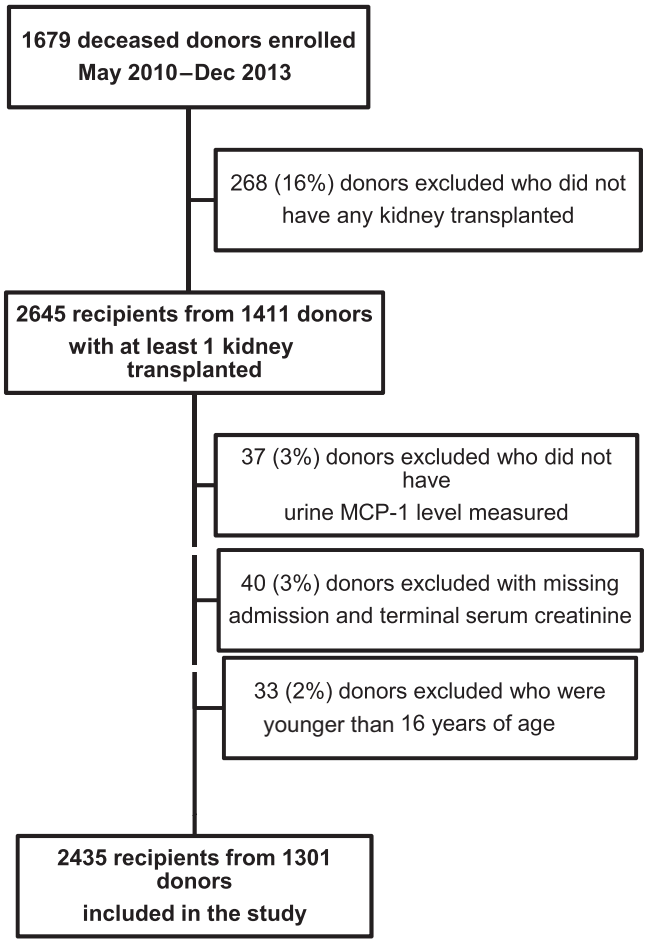

Figure 1. Enrollment of deceased kidney donors and recipients in the study cohort. Dec, December; MCP-1, monocyte chemoattractant protein-1.

Table 1. Deceased donor characteristics, stratified by acute kidney injury status

Characteristics	All ($N = 1301$)	No AKI ($n = 1190$)	AKI ($n = 111$)	P value[a]
Age, yr	41.45 ± 14.53	41.66 ± 14.66	39.2 ± 12.91	0.094
Male sex	785 (60)	718 (60)	67 (60)	0.996
Black race	206 (16)	184 (15)	22 (20)	0.229
Height, cm	171.17 ± 10.81	171.11 ± 10.91	171.83 ± 9.72	0.689
Weight, kg	83.32 ± 22.09	82.98 ± 21.43	87 ± 28.17	0.315
Hypertension	399 (31)	363 (31)	36 (32)	0.673
Diabetes	130 (10)	120 (10)	10 (9)	0.718
Cause of death				
Stroke	428 (33)	395 (33)	33 (30)	0.069
Anoxia	427 (33)	379 (32)	48 (43)	
Head trauma	396 (30)	368 (31)	28 (25)	
Other	50 (4)	48 (4)	2 (2)	
Hepatitis C virus seropositive	48 (4)	43 (4)	5 (5)	0.634
Admission serum creatinine, mg/dl	1.1 ± 0.61	1.1 ± 0.6	1.03 ± 0.64	0.010
Terminal serum creatinine, mg/dl	1.17 ± 0.85	1.01 ± 0.46	2.93 ± 1.68	<0.001
Admission creatinine > terminal creatinine	624 (48)	624 (52)	0 (0)	<0.001
Extended criteria donor[b]	246 (19)	226 (19)	20 (18)	0.802
Donation after circulatory death	206 (16)	193 (16)	13 (12)	0.214
Kidney donor risk index	1.29 ± 0.41	1.28 ± 0.42	1.36 ± 0.36	0.010
Kidney donor profile index, %	48.23 ± 27.33	47.63 ± 27.71	54.75 ± 21.95	0.010
No. of kidneys transplanted				
1	167 (13)	139 (12)	28 (25)	<0.001
2	1134 (87)	1051 (88)	83 (75)	

AKI, acute kidney injury. AKI refers to at least a 2-fold increase in serum creatinine from admission to the terminal value (AKI stage 2 or higher).
[a]Wilcoxon rank sum test for continuous variables and χ^2 test for categorical variables.
[b]Extended criteria donor was defined as a donor older than 60 years of age or a donor older than 50 years of age with 2 of the following: a history of high blood pressure, creatinine level ≥1.5, or death resulting from a stroke.
Values reported are mean ± SD or *n* (%).

uMCP-1 and Recipient Delayed Graft Function

Delayed graft function (DGF) developed in a total of 756 recipients (31%). Recipient characteristics stratified by DGF status are shown in Table 3. Median (interquartile range) donor uMCP-1 concentrations were significantly higher in recipients with DGF compared with recipients without DGF (0.43 [0.18–1.17] ng/ml vs. 0.31 [0.11–0.82] ng/ml, $P < 0.001$), as shown in Supplementary Figure S4. A histogram distribution is also shown in Supplementary Figure S5. As shown in Table 4, tertiles of uMCP-1 were weakly associated with DGF in unadjusted analysis.

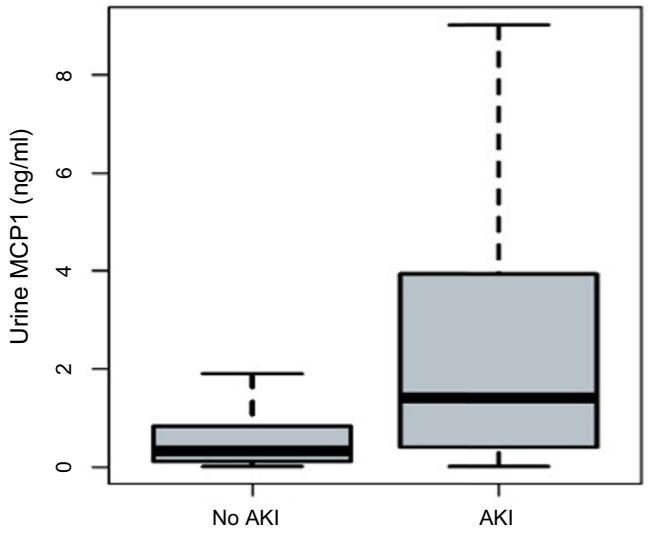

Figure 2. Donor urine monocyte chemoattractant protein-1 (uMCP-1) concentrations stratified by donor acute kidney injury (AKI) status. Box plot shows the 25th, 50th (median), and 75th percentile values for donor uMCP-1 by donor AKI status. The median values for the no AKI and the AKI group are 0.32 ng/ml and 1.35 ng/ml ($P < 0.001$), respectively. Outliers are not represented in this plot; 195 outliers (urine MCP1 ≥1.93 ng/ml) for the no AKI group were suppressed, and 29 outliers (urine MCP1 ≥9.24 ng/ml) for the AKI group were suppressed. Of note, outliers were included in all analyses and were only excluded for the visual representation of this box plot.

Given evidence that AKI modified the effect of uMCP-1 concentrations on recipient DGF ($P_{\text{interaction}} = 0.01$), we stratified the analysis by donor AKI status. In donor kidneys without AKI, second tertile uMCP-1 values were independently associated with a 23% increased risk of recipient DGF compared with uMCP-1 values in the first tertile. In donor kidneys with AKI, there was no significant association between uMCP-1 tertiles and recipient DGF in unadjusted and adjusted analyses. Donor uMCP-1 levels when indexed to urine creatinine concentration did not affect the estimates of the outcomes as shown in Supplementary Table S3.

Table 2. Association of uMCP-1 with risk of donor AKI

Log_2 uMCP-1/tertiles of uMCP-1 (range in ng/ml)	N[a]	Rate of AKI, N (%)[b]	Relative risk (95% CI) for AKI	
			Unadjusted	Adjusted[c]
T1 (<0.19)	433	8 (2)	1.0 (referent)	1.0 (referent)
T2 (0.19–0.63)	434	29 (7)	**3.62 (1.67–7.82)**	**3.72 (1.73–8.03)**
T3 (0.64–16.90)	434	74 (17)	**9.23 (4.50–18.91)**	**9.80 (4.75–20.23)**
Log uMCP-1	1301	111 (9)	**1.50 (1.39–1.61)**	**1.54 (1.42–1.67)**

AKI, acute kidney injury; CI, confidence interval; T, tertile; uMCP-1, urine monocyte chemoattractant protein-1.
[a]Number of donors in each tertile and total number of donors for log-transformed MCP-1.
[b]Percentage of donors in each tertile with AKI stage 2 or higher and percentage of donors in the entire cohort with AKI stage 2 or higher for log-transformed MCP-1.
[c]Adjusted for the following donor variables that comprise the kidney donor risk index (except terminal serum creatinine): age (years), height (cm), weight (kg), black race, history of hypertension, history of diabetes, stroke as cause of death, and donation after cardiac determination of death status.
AKI refers to at least a 2-fold increase in serum creatinine from admission to the terminal value (AKI stage 2 or higher). Bold text represents statistically significant values ($P < 0.05$).

Table 3. Kidney transplant recipient characteristics, stratified by delayed graft function (DGF) status

Characteristic	All ($N = 2435$)	Non-DGF ($n = 1679$)	DGF ($n = 756$)	P value[a]
Age, yr	52.92 ± 14.83	52.06 ± 15.58	54.81 ± 12.84	0.001
Male sex	1493 (61)	997 (59)	496 (66)	0.004
Black race	959 (39)	588 (35)	371 (49)	<0.001
Hispanic ethnicity	279 (11)	200 (12)	79 (10)	0.295
Cause of ESRD				
Diabetes	718 (29)	487 (29)	231 (31)	0.034
Hypertension	657 (27)	430 (26)	227 (30)	
Other or unknown	504 (21)	368 (22)	136 (18)	
Glomerulonephritis	394 (16)	285 (17)	109 (14)	
Graft failure	162 (7)	109 (6)	53 (7)	
HLA mismatch level				
0	153 (6)	126 (8)	27 (4)	<0.001
1	21 (1)	10 (1)	11 (1)	
2	83 (3)	63 (4)	20 (3)	
3	292 (12)	201 (12)	91 (12)	
4	644 (26)	452 (27)	192 (25)	
5	822 (34)	552 (33)	270 (36)	
6	414 (17)	270 (16)	144 (19)	
Panel reactive antibodies (%)				
0	1549 (64)	1048 (62)	501 (66)	0.147
1–20	178 (7)	119 (7)	59 (8)	
21–80	326 (13)	237 (14)	89 (12)	
>80	382 (16)	275 (16)	107 (14)	
Cold ischemia time, h	15.28 ± 7.09	14.42 ± 6.9	17.21 ± 7.14	<0.001
Preemptive transplant	274 (11)	246 (15)	28 (4)	<0.001
Serum creatinine at transplant, mg/dl	7.76 ± 3.35	7.46 ± 3.36	8.44 ± 3.22	<0.001

DGF, delayed graft function; ESRD, end-stage renal disease; HLA, human leukocyte antigen.
[a]Wilcoxon rank sum test for continuous variables and χ^2 test for categorical variables. Values reported are mean ± SD or *n* (%).

uMCP-1 and 6-Month eGFR

A total of 2311 recipients (95%) had 6-month eGFR data available for analyses. We imputed 6-month eGFR values in 124 recipients (5%) due to death or graft failure before 6 months. The mean (SD) 6-month eGFR was 56 (23.8) ml/min per 1.73 m^2. The Pearson correlation between uNGAL and uMCP-1 was $r = 0.65$, $P < 0.001$. As shown in Table 5, after adjusting for uNGAL in the final model, each doubling of donor uMCP-1 concentration was independently associated with a modest increase in the recipient 6-month eGFR (by 0.81 [0.21–1.41] ml/min per 1.73 m^2).

The distribution of the 6-month eGFR by DGF status is shown in Supplementary Figure S6. Given evidence that DGF modified the association between uMCP-1 concentrations and 6-month eGFR ($P_{\text{interaction}} = 0.03$), we stratified the analysis by DGF status. In recipients who did not experience DGF, each doubling of donor uMCP-1 was associated with an increased adjusted 6-month eGFR of 0.77 (0.10–1.45) ml/min per 1.73 m^2. In recipients with DGF, each doubling of donor uMCP-1 was associated with a 0.82 (0.0014–1.64) ml/min per

Table 4. Association between uMCP-1 and delayed graft function (DGF)

Log_2 uMCP-1/tertiles of uMCP-1	*N* (%)[a]	Relative risk (95% CI) of DGF			
		Unadjusted	Adjusted for donor variables only[b]	Adjusted for donor, transport, and recipient variables[c]	Adjusted for donor, transport, recipient, and donor uNGAL[d]
All recipients					
Log uMCP-1		**1.10 (1.07–1.13)**	**1.07 (1.04–1.11)**	1.02 (0.99–1.05)	0.99 (0.95–1.03)
T1	811 (24)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
T2	813 (33)	**1.39 (1.18–1.65)**	**1.29 (1.10–1.51)**	**1.26 (1.08–1.48)**	**1.20 (1.02–1.42)**
T3	811 (36)	**1.53 (1.29–1.80)**	**1.35 (1.15–1.59)**	1.10 (0.93–1.30)	1.00 (0.82–1.21)
P value[e]		<0.001	0.018	0.012	0.018
No donor AKI (stage 1 or no AKI)					
Log uMCP-1		**1.08 (1.04–1.12)**	**1.05 (1.01–1.08)**	1.01 (0.98–1.05)	0.99 (0.94–1.03)
T1	799 (23)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
T2	765 (32)	**1.39 (1.17–1.66)**	**1.27 (1.08–1.50)**	**1.29 (1.10–1.52)**	**1.23 (1.04–1.46)**
T3	677 (33)	**1.42 (1.19–1.70)**	**1.20 (1.01–1.43)**	1.08 (0.91–1.29)	0.99 (0.81–1.21)
P value[e]		<0.001	0.018	0.006	0.007
Donor AKI (stage 2 or higher)					
Log uMCP-1		1.05 (0.98–1.12)	1.03 (0.96–1.10)	1.00 (0.93–1.08)	1.00 (0.91–1.09)
T1	12 (67)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
T2	48 (48)	0.71 (0.44–1.14)	0.76 (0.48–1.20)	0.76 (0.47–1.24)	0.73 (0.45–1.19)
T3	134 (64)	0.78 (0.51–1.19)	0.81 (0.54–1.22)	0.81 (0.52–1.26)	0.76 (0.46–1.23)
P value[e]		0.438	0.317	0.574	0.485

AKI, acute kidney injury; CI, confidence interval; DGF, delayed graft function; T, tertile; uMCP-1, monocyte chemoattractant protein-1; uNGAL, urinary neutrophil gelatinase-associated lipocalin.
[a]Number of recipients in each tertile. Values in parentheses represent the percentage of recipients in each tertile with DGF.
[b]Donor variables used for adjustment: age (years), height (cm), weight (kg), black race, history of hypertension, history of diabetes, stroke as cause of death, terminal serum creatinine (mg/dl), and donation after cardiac determination of death status.
[c]Includes all variables listed plus cold ischemia time (hours), use of machine perfusion, and the following recipient variables: age (years), black race, sex, previous kidney transplant, diabetes as the cause of end-stage renal disease, duration of end-stage renal disease (months), number of human leukocyte antigen mismatches, body mass index (kg/m^2), and panel reactive antibody (%).
[d]Includes all variables listed plus log2-transformed donor uNGAL.
[e]All type 3 *P* values result from a χ^2 test with 2 degrees of freedom.
The range (ng/ml) of MCP-1 level per tertile is as follows: T1 (<0.19), T2 (0.19–0.63), and T3 (0.64–16.90). Bold text represents statistically significant values with $P < 0.05$. The interaction between uMCP-1 tertiles and AKI was significant ($P = 0.01$).

1.73 m^2 higher 6-month eGFR adjusted for donor, transport, and recipient characteristics. However, the association was no longer statistically significant after further adjustment for donor uNGAL. There were no significant associations between donor uMCP-1 tertiles and recipient 6-month eGFR. uMCP-1 concentrations indexed to urine creatinine had results similar to raw values, as shown in Supplementary Table S4.

uMCP-1 and Graft Failure

There were 228 cases of graft failure over a median recipient follow-up time of 2.35 years (interquartile range, 1.70–3.09 years). As shown in Table 6, there were no significant associations between donor uMCP-1 and graft failure in all recipients or when stratified by DGF. (There was no significant evidence that DGF modified the association between log uMCP-1 and graft failure, $P_{\text{interaction}} = 0.601$.)

Perfusate MCP-1 Concentrations

A total of 955 kidneys (39%) were placed on HMP and subsequently transplanted, and in 365 of these kidneys (38%) DGF developed. The median (interquartile range) MCP-1 concentration was lower in base compared with post perfusate samples (0.023 [0.014–0.061] ng/ml vs. 0.082 [0.036–0.199] ng/ml, $P < 0.0001$). As shown in Table 7, recipients with DGF had significantly higher base and post perfusate MCP-1 concentrations. There were no independent associations, however, for perfusate MCP-1 with recipient DGF or 6-month eGFR in multivariable analyses (Supplementary Table S5).

DISCUSSION

In an effort to improve current prediction tools for graft function at the time of deceased donor nephrectomy, we investigated associations between deceased-donor uMCP-1, a chemokine involved in inflammation and repair after kidney injury, and kidney allograft outcomes. Donor uMCP-1 concentration was strongly associated with donor AKI in a dose-dependent manner, demonstrating that the release of this protein and associated inflammation are closely linked to renal injury. In addition, the increase in perfusate MCP-1 concentrations from base to post time points provides evidence of ongoing release during HMP, possibly from donor macrophages still residing within the kidney after procurement. Donor uMCP-1 levels were also associated with modestly improved allograft function at 6 months after transplantation in those without DGF, which suggested that MCP-1 may

Table 5. Association between uMCP-1 and 6-month eGFR

	β coefficient (95% CI) for 6-month eGFR			
Log_2 uMCP-1/tertiles of uMCP-1	Unadjusted	Adjusted for donor variables only[a]	Adjusted for donor, transport, and recipient variables[b]	Adjusted for donor, transport, recipient variables, and donor uNGAL[c]
All recipients				
Log uMCP-1	−0.17 (−0.67 to 0.33)	−0.17 (−0.61 to 0.27)	0.28 (−0.19 to 0.75)	**0.81 (0.21–1.41)**
T1	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
T2	−1.78 (−4.50 to 0.94)	−1.16 (−3.43 to 1.11)	−0.61 (−2.86 to 1.64)	0.21 (−2.20 to 2.61)
T3	−1.29 (−3.95 to 1.36)	−1.19 (−3.47 to 1.09)	0.61 (−1.76 to 2.99)	2.14 (−0.69 to 4.98)
P value[d]	0.404	0.685	0.609	0.254
Recipients without DGF				
Log uMCP-1	−0.23 (−0.80 to 0.33)	−0.29 (−0.80 to 0.23)	0.10 (−0.45 to 0.64)	**0.77 (0.10–1.45)**
T1	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
T2	−0.32 (−3.29 to 2.65)	−0.54 (−3.10 to 2.02)	−0.14 (−2.68 to 2.39)	1.21 (−1.53 to 3.95)
T3	−1.25 (−4.21 to 1.70)	−1.68 (−4.28 to 0.92)	−0.06 (−2.78 to 2.67)	2.23 (−0.97 to 5.44)
P value[d]	0.735	0.915	0.997	0.374
Recipients with DGF				
Log uMCP-1	0.98 (0.13–1.83)[d]	0.53 (−0.22 to 1.28)	0.82 (0.00–1.64)[d]	0.78 (−0.37 to 1.93)
T1	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
T2	−1.46 (−6.06, 3.14)	−0.44 (−4.51 to 3.64)	0.08 (−4.08 to 4.23)	−0.23 (−4.57 to 4.11)
T3	3.27 (−1.39 to 7.94)	2.05 (−2.13 to 6.23)	3.05 (−1.35 to 7.44)	2.32 (−3.08 to 7.72)
P value[d]	0.086	0.422	0.291	0.546

CI, confidence interval; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; uMCP-1, monocyte chemoattractant protein-1; uNGAL, urinary neutrophil gelatinase-associated lipocalin.
[a]Donor variables used for adjustment: age (years), height (cm), weight (kg), black race, history of hypertension, history of diabetes, stroke as cause of death, terminal serum creatinine (mg/dl), and donation after cardiac determination of death status.
[b]Includes donor variables listed plus cold ischemia time (hours), use of machine perfusion, and the following recipient variables: age (years), black race, sex, previous kidney transplant, diabetes as the cause of end-stage renal disease, duration of end-stage renal disease (months), number of human leukocyte antigen mismatches, body mass index (kg/m^2), and panel reactive antibody (%).
[c]Includes all variables listed plus log_2-transformed donor uNGAL.
[d]All type 3 *P* values result from a χ^2 test with 2 degrees of freedom.
The range (ng/ml) of MCP-1 level per tertile is as follows: T1 (<0.19), T2 (0.19–0.63), and T3 (0.64–16.90). Bold text represents statistically significant values with $P < 0.05$. The interaction between log_2 uMCP-1 and DGF was significant ($P = 0.03$).

play an adaptive role in renal recovery after clinical ischemia-reperfusion injury. However, this association was not significant when assessing graft failure on follow-up. Based on our overall results, the role of donor uMCP-1 in predicting subsequent recipient graft function is of minimal clinical utility.

In this comprehensive study, we adjusted for several donor, recipient, and transport characteristics. We also adjusted for donor uNGAL to account for the severity of nephron injury as repair markers will be released in response to ongoing injury. Given the moderate correlation between NGAL and MCP-1, we postulated that with an increase in inflammation and injury, there is a need for more repair as shown by an increase in the concentration of MCP-1.

It is important to note that current allograft quality assessment tools are limited in their ability to reliably predict subsequent allograft function, possibly due to continued reliance on terminal serum creatinine and other donor clinical characteristics that relate poorly to biological processes (e.g., inflammation and repair) that influence graft outcomes. The limitations of serum creatinine are well-known given its delayed increase after renal injury and variability related to nonrenal factors such as diet, muscle mass, and liver function.[19–21] In contrast, NGAL is rapidly released by injured renal tubular epithelial cells, especially following ischemia-reperfusion, and has been shown to be an effective AKI biomarker.[22–24] However, both donor uNGAL and liver-type fatty acid binding protein showed only a modest association with recipient graft function, which led us to evaluate additional biological processes initiated in the donor at organ procurement (e.g., repair) that may affect recipient outcomes.[3] Despite the biological association of MCP-1 with the process of repair, we found no independent association with DGF, only a modest association with improved 6-month eGFR, and no significant associations with graft failure that may aid in reliably predicting allograft function. Considering the finding of no significant association between donor uMCP-1 and DGF, we suspect that the definition of DGF as any dialysis within the first week posttransplantation may have led to some misclassification of true ischemia reperfusion injury–induced DGF. For example, even in the absence of severe ischemia-reperfusion injury, perioperative electrolyte derangements can result in the need for a single dialysis session.[25] We also speculate that the finding of an independent association between donor uMCP-1 and 6-month allograft function only in those without DGF could be because the protection provided by donor uMCP-1 is more apparent (and possibly more effective)

Table 6. Association between uMCP-1 and graft failure

Log_2 uMCP-1/tertiles of uMCP-1	*N* (%)[a]	Relative risk (95% CI) of graft failure			
		Unadjusted	Adjusted for donor variables only[b]	Adjusted for donor, transport, and recipient variables[c]	Adjusted for donor, transport, recipient, and donor uNGAL[d]
All recipients					
Log uMCP-1		1.01 (0.95–1.07)	1.01 (0.95–1.07)	1.01 (0.94–1.08)	1.01 (0.93–1.10)
T1	811 (8)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
T2	813 (10)	1.23 (0.90–1.70)	1.22 (0.89–1.68)	1.24 (0.89–1.71)	1.27 (0.90–1.80)
T3	811 (9)	1.12 (0.81–1.54)	1.11 (0.79–1.57)	1.16 (0.82–1.63)	1.22 (0.81–1.82)
P value[e]		<0.001	0.018	0.012	0.018
No DGF					
Log uMCP-1		1.00 (0.93–1.08)	1.03 (0.94–1.12)	1.03 (0.94–1.13)	1.04 (0.93–1.17)
T1	618 (6)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
T2	545 (8)	1.27 (0.83, 1.95)	1.36 (0.88–2.10)	1.36 (0.87–2.12)	1.40 (0.87–2.27)
T3	516 (6)	0.99 (0.62, 1.58)	1.09 (0.65–1.81)	1.18 (0.71–1.96)	1.24 (0.70–2.20)
P value[e]		<0.001	0.018	0.006	0.007
DGF					
Log uMCP-1		0.96 (0.89–1.04)	0.97 (0.89–1.06)	0.98 (0.89–1.07)	0.99 (0.88–1.11)
T1	193 (16)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
T2	268 (15)	0.99 (0.64–1.55)	1.00 (0.65–1.56)	1.01 (0.64–1.58)	1.05 (0.66–1.68)
T3	295 (15)	0.97 (0.62–1.49)	1.06 (0.68–1.67)	1.08 (0.69–1.70)	1.21 (0.70–2.09)
P value[e]		0.438	0.317	0.574	0.485

CI, confidence interval; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; T, tertile; uMCP-1, urine monocyte chemoattractant protein-1; uNGAL, urinary neutrophil gelatinase-associated lipocalin. The interaction between log uMCP-1 and DGF was nonsignificant ($P = 0.601$) and uMCP-1 tertiles and DGF was also nonsignificant ($P = 0.605$).
[a]Number of recipients in each tertile. Values in parenthesis represent the percentage of recipients with graft failure per tertile.
[b]Donor variables used for adjustment: age (years), height (cm), weight (kg), black race, history of hypertension, history of diabetes, stroke as cause of death, terminal serum creatinine (mg/dl), and donation after cardiac determination of death status.
[c]Includes all variables listed plus cold ischemia time (hours), use of machine perfusion, and the following recipient variables: age (years), black race, sex, previous kidney transplant, diabetes as the cause of end-stage renal disease, duration of end-stage renal disease (months), number of human leukocyte antigen mismatches, body mass index (kg/m^2), and panel reactive antibody (%).
[d]Includes all variables listed above plus log_2-transformed donor uNGAL.
[e]All type 3 *P* values result from a χ^2 test with 2 degrees of freedom.
The range (ng/ml) of MCP-1 level per tertile is as follows: T1 (<0.19), T2 (0.19–0.63), and T3 (0.64–16.90).

in "healthier" kidneys that are able to recover rapidly from ischemia-reperfusion injury and avoid DGF. However, this association was no longer significant when assessing graft failure. It is possible that several other clinical events such as hospitalization, rejection, and infection confound the signal from donor MCP-1. It is also possible that donor MCP-1 is part of a larger complex panel of conventional, injury, and repair biomarkers, which, together, are able to predict recipient graft outcomes.

Our findings should be considered in the context of previous literature. MCP-1 has been linked with macrophage recruitment and renal fibrosis as a downstream effect of injury.[26] Our novel finding of improved 6-month eGFR in recipients without DGF in the setting of higher donor uMCP-1 concentration seems inconsistent with these previous reports of MCP-1 leading to renal disease progression. Some unique aspects of our study design might explain this apparent discrepancy. We tested donor rather than recipient uMCP-1 to predict allograft outcomes. We postulate that in this setting, exogenous delivery of (donor) MCP-1 to the recipient via the renal allograft has unique downstream effects compared with endogenous recipient MCP-1 production. Experimental data suggest that administering MCP-1 beyond a certain threshold activates a negative feedback pathway that reduces endogenous MCP-1

Table 7. Perfusate MCP-1 concentration stratified by delayed graft function (DGF) status

Biomarker	All ($N = 955$)	No DGF ($n = 590$)	DGF ($n = 365$)	*P* value[a]
Base MCP-1, ng/ml	0.023 (0.014–0.061)[b] $N = 636$	0.020 (0.014–0.052) $n = 420$	0.027 (0.014–0.085) $n = 149$	0.018
Post MCP-1, ng/ml	0.082 (0.036–0.199)[b] $N = 588$	0.072 (0.034– 0.169) $n = 381$	0.094 (0.040–0.240) $n = 207$	0.038
Delta MCP-1, ng/ml	0.043 (0.016–0.125) $N = 560$	0.039 (0.015–0.112) $n = 364$	0.051 (0.018–0.152) $n = 196$	0.051

[a]Wilcoxon rank-sum test (DGF vs. non-DGF).
[b]$P < 0.0001$ for difference in base and post based on Wilcoxon signed rank test.
Values are median (interquartile range). DGF, delayed graft function; MCP-1, monocyte chemoattractant protein-1. Delta is the difference in post minus base values. Base samples were taken within minutes of starting perfusion. Post samples were obtained before the organ procurement organization transferred management of the kidney.

production.[27] This might explain why donor MCP-1 concentrations are associated with favorable outcomes compared with recipient MCP-1 concentrations, which have been shown to be associated with renal function decline and allograft loss.[28] It is also possible that the protective effect that we observed between donor uMCP-1 and the 6-month eGFR is secondary to ischemic preconditioning rather than a direct effect of MCP-1.[29] Hence, renal allograft response to elevated donor uMCP-1 concentrations might elicit a reparative pathway that better adapts the allograft to subsequent ischemia-reperfusion injury.

These results are in agreement with those of our previous work and demonstrate that donor AKI does not preclude favorable recipient allograft outcomes.[30] However, donor MCP-1 was not associated with graft failure over a median follow-up of 2.35 years, which could be due to ongoing complex interplay of various biological processes beyond what is expressed by a single uMCP-1 measurement at the time of organ procurement. Hence multiple biomarkers as well as multiple measurements may be needed to discern these processes and delineate kidney transplant outcomes. Therefore, MCP-1 may play a role in multibiomarker allograft quality assessment, but, given that its association with 6-month eGFR is modest and there were no associations with graft failure, MCP-1 does not appear to have clinical utility as a standalone test in this setting. Nonetheless, further studies are needed to assess whether donor uMCP-1 may be a good indicator of longer term outcomes beyond 6 months.

Our study has limitations that should be considered. There may be unmeasured confounders related to inflammatory and alloimmune responses, which may further affect the association between donor uMCP-1 and recipient outcomes. Our study protocol does not capture posttransplant recipient biomarker data as there was no opportunity to obtain informed consent from recipients to collect samples across several transplantation centers. Thus, we could not determine the trajectory of uMCP-1 concentrations in the recipient. Also, observational deceased-donor studies such as the current cohort study lack transplant outcome information for discarded kidneys, indicating the potential for unavoidable selection bias.

In conclusion, we have demonstrated that higher concentrations of deceased-donor uMCP-1 are independently associated with donor AKI. However, a higher donor uMCP-1 concentration was only modestly associated with improved graft function at 6 months in recipients in whom DGF did not develop and no significant associations were found with graft failure on follow-up. The clinical utility and current role of donor uMCP-1 as a prognostic tool in predicting recipient graft outcomes are limited based on our results.

DISCLOSURE

All the authors declared no competing interests.

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Study Registration: ClinicalTrials.gov NCT01848249.

SUPPLEMENTARY MATERIAL

Table S1. Full model for the outcome of delayed graft function (DGF).

Table S2. Full model for the outcome of 6-month estimated glomerular filtration rate (eGFR).

Table S3. Association between $\log_2$ (urine monocyte chemoattractant protein-1 [MCP-1]/urine creatinine) and delayed graft function (DGF) defined as requiring dialysis within the first week posttransplantation.

Table S4. Association between $\log_2$ (urine monocyte chemoattractant protein-1 [MCP-1]/urine creatinine) and 6-month eGFR.

Table S5. Association between $\log_2$ perfusate monocyte chemoattractant protein-1 (MCP-1) (base, post, delta) and 6-month estimated glomerular filtration rate (eGFR).

Figure S1. Donor urine monocyte chemoattractant protein-1 (MCP-1) concentrations stratified by donor acute kidney injury (AKI) status.

Figure S2. Histogram distribution of donor urine monocyte chemoattractant protein-1 (MCP-1) levels across the entire cohort.

Figure S3. Histogram distribution of donor urine monocyte chemoattractant protein-1 (MCP-1) levels stratified by donor acute kidney injury (AKI) status.

Figure S4. Donor urine monocyte chemoattractant protein-1 (MCP-1) concentrations stratified by donor delayed graft function (DGF) status.

Figure S5. Histogram distribution of donor urine monocyte chemoattractant protein-1 (MCP-1) levels stratified by delayed graft function (DGF) status.

Figure S6. Distribution of 6-month estimated glomerular filtration rate by delayed graft function (DGF) status.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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