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Donor-derived Cell-free DNA as a Graft Injury Marker Following Kidney Transplantation

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Initial poor graft function after kidney transplantation (KTX) is commonly because of ischemia-reperfusion injury and may later manifest as premature graft loss.[1] The time frame of the ultimate outcome varies, and no biomarkers exist that risk stratify patients who are more likely to experience inferior graft outcomes. Donor-derived cell-free DNA (dd-cfDNA) is a molecular marker of active graft injury and may be valuable to predict the extent of tissue damage. Higher plasma fractions have been seen in recipients of deceased-donor compared with living-donor grafts within day 1[2] and day 5[3] post-KTX, presumably because of ischemia-reperfusion damage.

To examine the clinical validity of dd-cfDNA as a graft injury sensor, a retrospective study of all adults who underwent deceased-donor KTX between February 2019 and March 2020 at our center (n = 135) and with surveillance dd-cfDNA (AlloSure, CareDx, Brisbane, CA) at post-KTX days 14 to 37 (n = 71) was conducted. None had detectable preformed donor-specific antibody, defined as mean fluorescence intensity >1500 by Luminex assay. Follow-up was 1 y minimum. Basic immunosuppressive regimens were used and typically consisted of induction with antithymocyte globulin and corticosteroids (variable withdrawal posttransplant), and maintenance therapy with tacrolimus and mycophenolate sodium. Graft biopsies were performed for cause. Using SAS V.9.4 (SAS Institute Inc., Cary, NC), comparisons between low (≤0.5%) and elevated (>0.5%) dd-cfDNA groupings were statistically evaluated via chi-square test for categorical variables, Wilcoxon rank-sum test for continuous variables, and log-rank test for time to all-cause graft survival. The institution review board approved this study.

The dd-cfDNA fraction was low in 33 recipients and elevated in 38. Donor and recipient characteristics were not significantly different between the groups, other than body mass index, where patients with lower dd-cfDNA measurements tended to have higher body mass index (Table 1). Elevated levels of dd-cfDNA did not differentiate outcomes of posttransplant (1) dialysis requirement in the first week, (2) days to serum creatinine <3 mg/dL, (3) 1-y estimated glomerular filtration rate ≤40 mL/min/1.73, (4) 90-d acute rejection, (5) 90-d donor-specific antibody, or (6) time to all-cause graft survival ($P = 0.726$). Acute rejection frequency and severity were greater in the dd-cfDNA >0.5% group.

A study limitation is potential ascertainment bias because patients with delayed graft function were more likely to have dd-cfDNA obtained. Also, the small sample and single-center experience limit generalizability but do reflect real-world experience. Our cutoff level may lack discriminatory value because mean dd-cfDNA has been shown to stabilize at 0.35%[4] or 0.46%[2] after transplantation.

In deceased-donor KTX recipients with dd-cfDNA obtained post-KTX day 14 to 37, we found that dd-cfDNA ≥0.5% was not associated with early graft outcomes. The null result may be because of low power of the study to detect differences. The doubling of acute rejection and greater severity of rejection seen in the dd-cfDNA ≥0.5% group suggests that further work is warranted. Considering a higher demarcation (0.74% or 1%) as is used for defining rejection may be more clinically useful to identify active ischemia-reperfusion injury.[5,6] Additionally, absolute quantification of dd-cfDNA[3] or higher baseline levels throughout the early posttransplant course[2] may yield superior discrimination of graft injury.

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TABLE 1.

Donor, recipient, and outcome data by dd-cfDNA group

		Median (interquartile range) or N (%)		
	Characteristic	**dd-cfDNA ≤0.5 (n = 33)**	**dd-cfDNA >0.5 (n = 38)**	***P***
Donor	Posttransplant day of dd-cfDNA detection	30 (5)	27 (6)	0.009
	Donor age, y	36 (15)	41 (23)	0.836
	Donor kidney function			0.501
	Terminal serum creatinine <2 mg/dL	25 (76.0)	24 (63.2)	
	Terminal serum creatinine ≥2 mg/dL	6 (18.8)	9 (23.7)	
	Acute dialysis	2 (6.3)	5 (13.2)	
	DCD	16 (48.5)	16 (42.1)	0.590
	Cold ischemia time ≥30 h	16 (48.5)	18 (47.4)	0.925
Recipient	Recipient age, y	54 (16)	55 (22)	0.327
	Recipient Black race	11 (33.3)	16 (42.1)	0.448
	Recipient male	23 (69.7)	27 (71.1)	0.901
	Recipient diabetes	18 (54.5)	16 (42.1)	0.295
	Recipient pretransplant chronic dialysis	27 (81.8)	29 (76.3)	0.571
	Recipient CPRA >0%	22 (35.5)	13 (29.0)	0.217
	Recipient HLA mismatch >3	24 (72.7)	30 (78.9)	0.540
	Recipient de novo kidney transplant	30 (90.9)	34 (89.5)	0.840
	Recipient BMI, kg/m^2	34 (9)	28 (6)	0.001
	Recipient transplant ureteral stent placed	2 (6.1)	4 (10.5)	0.679[a]
	Recipient EPTS 1%–20%	7 (21.2)	9 (23.7)	0.851
	Recipient EPTS 21%–80%	22 (66.7)	23 (60.5)	
	Recipient EPTS 81%–100%	4 (12.1)	6 (15.8)	
Outcome	Posttransplant dialysis within 1 wk (DGF)	24 (72.7)	25 (65.8)	0.528
	Posttransplant <5 d to serum creatinine <3 mg/dL	4 (12.5)	6 (16.7)	0.674
	Posttransplant 5–17 d to serum creatinine <3 mg/dL	11 (34.4)	9 (25.0)	
	Posttransplant >17 d to serum creatinine <3 mg/dL	17 (53.1)	21 (58.3)	
	Posttransplant 1-y eGFR[b] ≤40 mL/min/1.73^2	9 (28.1)	10 (29.4)	0.908[a]
	Posttransplant 90-d acute rejection[c]	2 (6.1)	5 (13.2)	0.438[a]
	Borderline lesion	2 (d26–d33)	1 (d19)	
	Acute cellular rejection Banff 1a	0	3 (d22–d23–d30)	
	Acute cellular rejection Banff 2a	0	1 (d28)	
	Posttransplant 90-d donor-specific antibody	1 (3.0)	1(2.6)	1.000[a]

[a]Fisher exact test.

[b]eGFR (CKD-EPI) = $141 \times \min(S_{Cr}/\kappa, 1)^{\alpha} \times \max(S_{Cr}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ (if female) × 1.159 (if Black).

[c]Acute rejection was defined using Banff schema and included borderline lesions (indicates days to rejection post–kidney transplantation).

BMI, body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CPRA, calculated panel reactive antibody; DCD, donation after circulatory death; dd-cfDNA, donor-derived cell-free DNA; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; EPTS, estimated posttransplant survival; S_{Cr}, serum creatinine.

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