Repeat Kidney Transplant Patients with Active Rejection Have Elevated Donor-Derived Cell-Free DNA

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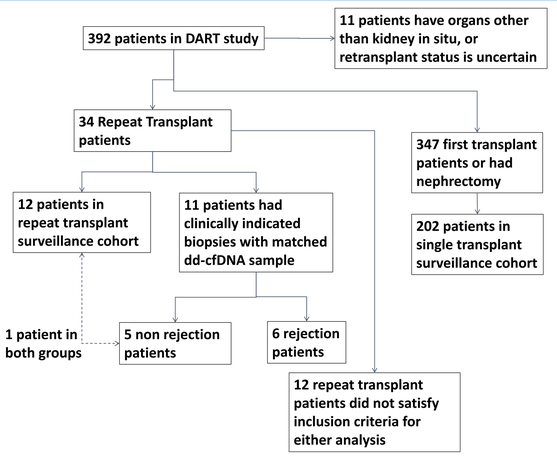
Introduction

Many (13% in 2015) kidney transplant patients receive repeat transplants.[1] The survival of repeat transplants are inferior to that of primary allografts.[1] Recipients of kidney repeat transplants are at high immunological risk for rejection and complications of immunosuppression,[2] and therefore these patients have high potential benefits from access to new biomarkers to monitor the status of the allograft. Donor-derived cell-free DNA test (dd-cfDNA, AlloSure®) has established performance characteristics to diagnose the probability of active rejection in *de novo* kidney transplant recipients.[3,4] dd-cfDNA is significantly higher (median 1.6%) in association with biopsy-based diagnosis of active rejection as compared to dd-cfDNA levels in cases of histology findings of no rejection (median 0.3%)[3]. Here we characterize dd-cfDNA in patients who have received a repeat kidney transplant where the prior graft remains *in situ*.

Methods

Plasma dd-cfDNA was collected from patients enrolled in the 14-center Circulating Donor-Derived Cell-free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients study (DART, Clinical Trials Identifier NCT02424227)[3,5]. The DART study included 34 patients with more than one renal allograft *in situ* (repeat transplants). Patients from the repeat transplant group who began dd-cfDNA surveillance testing within 2 months post-transplant (≤ 75 dpt) and had no clinically indicated biopsy at the first visit and no rejection anytime while on the study (n=12, repeat transplant surveillance cohort) and those who met the same conditions but had single kidney transplant (n=202, single transplant surveillance cohort) were included in the surveillance groups. Patient demographics and dd-cfDNA distribution were compared between the repeat and single transplant surveillance cohorts. A cohort with clinically indicated biopsy of the most recent allograft (n=11) was examined for the impact of biopsy-proven active rejection on dd-cfDNA in repeat transplant patients. dd-cfDNA was quantified in the CareDx CLIA laboratory using a clinical-grade targeted next generation sequencing method (AlloSure)[4]. The test computes the total dd-cfDNA contributed by all allografts, and does not distinguish which kidney(s) may have contributed dd-cfDNA.

Patients used in this study



Results

Table 1. Repeat and single transplant surveillance patients share similar demographics and clinical correlates.

Variable	All	Single transplant surveillance cohort	Repeat transplant surveillance cohort	*P*-value
Number of Patients	214	202	12	
Number of Visits	1207	1152	55	
Number of Visits per Patient	5.6	5.7	4.6	
Race				0.976
American Indian or Alaskan Native	1 (0 %)	1 (0 %)		
Asian	5 (2 %)	5 (2 %)	0 (0 %)	
Black or African American	70 (33 %)	65 (32 %)	0 (0 %)	
Hispanic/Latino	24 (11 %)	23 (11 %)	5 (42 %)	
Native Hawaiian/Other Pacific Islander	1 (0 %)	1 (0 %)	1 (8 %)	
Other	18 (8 %)	17 (8 %)	0 (0 %)	
White	95 (44 %)	90 (45 %)	1 (8 %)	
Male Sex	126 (59 %)	118 (58 %)	5 (42 %)	0.765
CMV Status				0.433
D-/R-	29 (14 %)	29 (14 %)	0 (0 %)	
D-/R+	54 (25 %)	52 (26 %)	2 (17 %)	
D+/R-	25 (12 %)	24 (12 %)	1 (8 %)	
D+/R+	84 (39 %)	77 (38 %)	7 (58 %)	
Unknown	22 (10 %)	20 (10 %)	2 (17 %)	
Donor Type				0.48
Deceased donor	145 (68 %)	137 (68 %)	8 (67 %)	
Living unrelated	36 (17 %)	35 (17 %)	1 (8 %)	
Living related	33 (15 %)	30 (15 %)	3 (25 %)	
DSA Positive patient	38 (18 %)	34 (17 %)	4 (33 %)	0.233
CMV Infection	18 (8 %)	17 (8 %)	1 (8 %)	1
BKV Infection	31 (14 %)	29 (14 %)	2 (17 %)	0.687
Age at Enrollment	50 ± 13	51 ± 12	40 ± 12	***0.002***
Days Post Transplant at Enrollment	36 ± 22	35 ± 20	49 ± 44	***0.027***
Weight	82 ± 21	83 ± 21	71 ± 11	0.123
Height	172 ± 11	172 ± 11	170 ± 11	0.606
Creatinine	1.9 ± 1.3	1.9 ± 1.3	1.8 ± 0.7	0.883
EGFR	46 ± 17	45 ± 17	47 ± 16	0.803
HLA Class 1 Mismatches	2.9 ± 1.1	2.9 ± 1.1	2.3 ± 1.2	0.059
HLA Class 2 Mismatches	1.2 ± 0.7	1.2 ± 0.7	1.2 ± 0.8	0.892

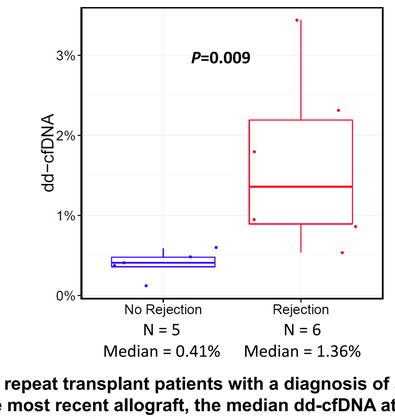
The demographics of the repeat and single transplant surveillance cohort are similar, with the repeat transplant cohort being slightly younger and enrolled slightly later post transplant than the single transplant cohort.

Table 2. Distribution of dd-cfDNA levels in repeat and single transplant surveillance patients.

	Single transplant surveillance cohort	Repeat transplant surveillance cohort
# Pts (# Samples)	202 (1152)	12 (55)
# Samples / Pt	5.7	4.6
Median [25th, 75th]	0.19% [0.10%, 0.35%]	0.29% [0.12%, 0.68%]
% Samples > 1%	5.5%	10.9%
% Samples > 0.2%	48.2%	60.0%
Maximum	6.56%	2.86%
P (difference in dd-cfDNA in single and repeat transplant)	< 0.001	

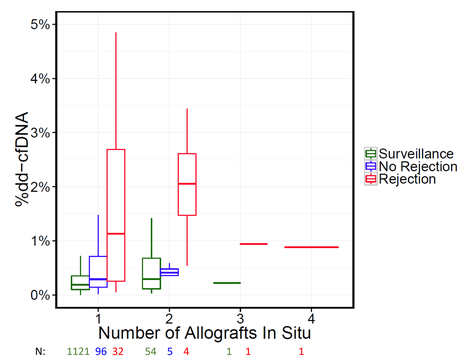
Median dd-cfDNA is slightly elevated in repeat transplant surveillance patients compared to single transplant surveillance patients. Both cohorts have dd-cfDNA levels that are significantly lower than the clinically demonstrated 1% threshold for rejection[3].

Figure 1. dd-cfDNA is higher in repeat transplant patients who have biopsy-proven rejection than those without rejection.



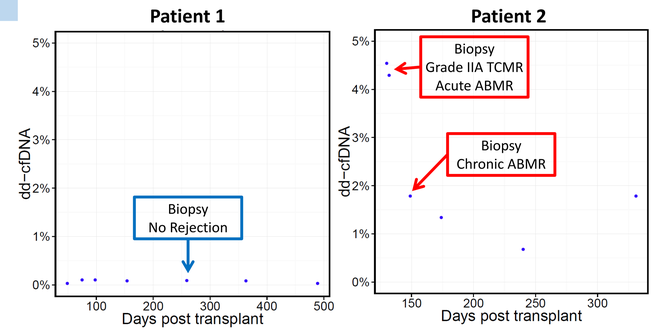
In six repeat transplant patients with a diagnosis of active rejection of the most recent allograft, the median dd-cfDNA at the time of a clinically indicated biopsy was 1.4%, significantly higher than the median 0.4% (*P*=0.009) in five repeat transplant patients negative for rejection at a clinically-indicated biopsy. These dd-cfDNA values are comparable to single transplant patients: those with active rejection had a median dd-cfDNA of 1.6%, and those with no biopsy findings of rejection had a median of 0.3%.[3]

Figure 2. dd-cfDNA is higher in patients with active rejection than in patients with no rejection, regardless of number of allografts in situ.



Patients with biopsy-proven rejection have increased levels of dd-cfDNA compared to patients with no biopsy-proven rejection and surveillance patient visits, regardless of the number of allografts.

Figure 3. Repeat transplant patient case studies.



Patient 1 (left) has two kidney allografts *in situ* and is in the repeat transplant surveillance cohort. Patient had low dd-cfDNA for 18 months post transplant (≤0.1%). Patient had one surveillance biopsy which showed no rejection.
Patient 2 (right) has two kidney allografts *in situ* and is in the repeat transplant rejection group. Patient had high dd-cfDNA (4.5%) and a biopsy confirmed rejection (Grade IIA TCMR and acute ABMR) at day 128 which was treated with pulse solumedrol and IVIG. Three weeks later dd-cfDNA was decreased to 1.8% and biopsy showed no TCMR and chronic ABMR. Patient was given solumedrol and dd-cfDNA continued to decrease.

Conclusions

- In the small cohort of surveillance patients with no biopsy-proven rejection, repeat kidney transplant patients had slightly higher, but clinically similar dd-cfDNA levels (median 0.29%) compared to single allograft patients (median 0.19%).
- The dd-cfDNA levels in repeat transplant patients with no rejection by biopsy are significantly lower than dd-cfDNA levels in either single or repeat transplant patients with biopsy-proven rejection.
- The dd-cfDNA levels in repeat transplant patients with biopsy-proven rejection are significantly higher than dd-cfDNA levels in either single or repeat transplant patients without biopsy-proven rejection.
- This dd-cfDNA test has the potential to be used to identify active rejection and related injury as well as monitor rejection treatment in patients with repeat kidney transplants.

References

[1] United States Renal Data System. 2017 *USRDS annual data report: Epidemiology of kidney disease in the United States.* National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2017.
[2] Alfonso et al.,2017. *Analysis of Risk Factors for Kidney Retransplant Outcomes Associated with Common Induction Regimens: A Study of over Twelve-Thousand Cases in the United States.* Journal of Transplantation
[3] Bloom et al., 2017. *Cell-Free DNA and Active Rejection in Kidney Allografts.* J Am Soc Nephrol.
[4] Grskovic et al., 2016. *Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients.* J Mol Diagn 18:890-902.
[5] Bromberg et al., 2017. *Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications.* J Applied Lab Medicine.

Disclosures

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DH, MG and JY are employees of CareDx.