Donor-Derived Cell-Free DNA Identifies Antibody-Mediated Rejection With Graft Injury in DSA-Positive Kidney Transplant Recipients

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Background

Antibody mediated rejection (ABMR) is an important cause of acute and chronic allograft dysfunction and graft loss.[1] Many transplant patients are monitored for donor-specific antibodies (DSA), which are a risk factor for ABMR.[2] Pre-formed or *de novo* donor-specific antibodies (DSAs) may lead to ABMR, but are not diagnostic alone.[3] Donor-derived cell-free DNA (dd-cfDNA) is a noninvasive test of allograft injury that may enable more frequent, quantitative, and safer assessment of allograft rejection and injury status. dd-cfDNA discriminates active rejection in kidney transplant patients indicated for biopsy,[4] with a greater discrimination for ABMR as compared to T cell mediated rejection.

Objective

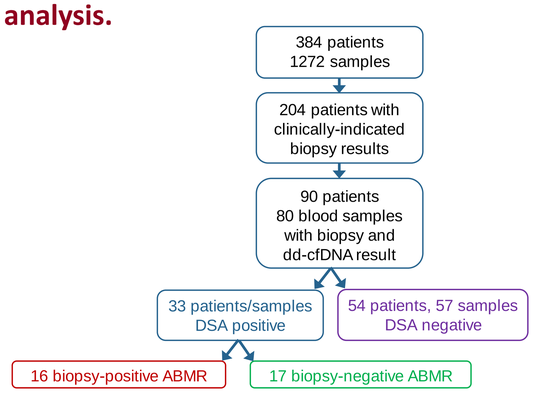
This study assesses the combined use of dd-cfDNA and DSAs to diagnose ABMR.

Methods

dd-cfDNA was assayed in 90 blood samples with paired clinically indicated biopsies from 87 kidney transplant patients (Figure 1). Patients were divided into those who had DSA (DSA+, n=33 samples) and those who did not (DSA–, n=57 samples). Prevalences of biopsy-based diagnosis of ABMR were computed. Samples were further divided into dd-cfDNA above 1% (dd-cfDNA+) and dd-cfDNA below 1% (dd-cfDNA–).

All patients were part of the DART study (a prospective, observational study at 14 centers) to assess the ability of dd-cfDNA to discriminate active rejection from no-rejection.[4] These analysis are based on the subset of patients with a clinically-indicated biopsy. The ABMR were defined according to Banff 2013 and comprised 11 ABMR and 5 mixed ABMR/TCMR. DSA positive vs. negative was determined by center protocol, generally using 1000 MFI as the threshold. dd-cfDNA was measured using an analytically and clinically validated assay, AlloSure®.[5] A dd-cfDNA cutoff of 1% was used to classify dd-cfDNA as positive or negative.[4,6] dd-cfDNA is measured by determining the fraction of donor-derived alleles at single-nucleotide polymorphism (SNP) locations.[5] The AlloSure assay does not require prior genotyping of the donor or recipient. SNPs are chosen that have two alleles, distributed approximately equally in the population; SNP regions are amplified from the low levels of dd-cfDNA found in plasma; then next-generation sequencing is used to count each allele.

Figure 1: Patients and samples from DART used in the current analysis.



Results

Table I: The patient population is similar in the DSA+ and DSA– groups.

	All	DSA+	DSA–	p value
Number of Patients	87	33	54	
Age	51 ± 14	47 ± 14	53 ± 13	0.024
Gender (% male)	60%	58%	61%	0.824
Race (% Caucasian)	53%	48%	56%	0.542
Donor Type				0.259
Deceased	60%	67%	56%	
Living unrelated	26%	15%	33%	
Living related	14%	18%	11%	

Figure 2: The median level of dd-cfDNA in samples from DSA+ patients with ABMR was significantly higher than the median level in DSA+ patients without ABMR.

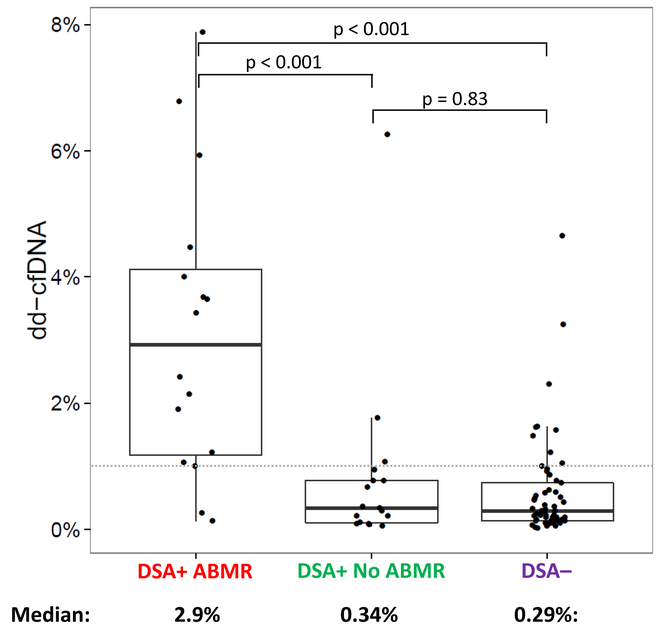


Table II: In DSA+ patients, the PPV is over 80% at a 1% threshold, and nearly 90% at 2.9% (median ABMR)

DSA used	dd-cfDNA cutoff	Sens	Spec	AUC ROC	PPV	NPV
✓	n/a	n/a	77%	n/a	**48%**	n/a
n/a	1%	81%	83%	87%	**44%**	96%
✓	1%	81% (67%, 100%)	82% (67%, 100%)	86% (70%, 98%)	**81%** (69%, 100%)	83% (73%, 100%)
✓	2.9%*	50% (30%, 70%)	94% (88%, 100%)	86% (70%, 98%)	**89%** (75%, 100%)	68% (60%, 77%)

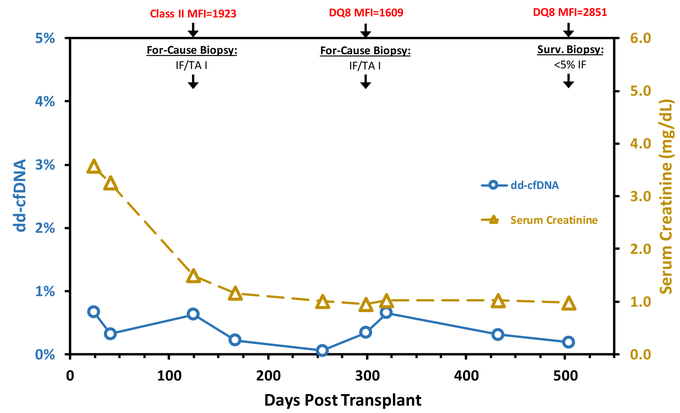
* 2.9% used since it is the median dd-cfDNA of ABMR samples in DART. 95% confidence intervals shown in parentheses.

Conclusions

- Patients with dd-cfDNA+/DSA+ results have a high probability of active ABMR
- Patients with dd-cfDNA–/DSA+ results are unlikely to have ABMR
- The combined use of dd-cfDNA and DSA testing may improve the non-invasive diagnosis of active ABMR in kidney transplant patients. Further studies will be performed to continue to validate these results.

Case Study: Despite DSA above 1000 MFI, there is no ABMR (or TCMR) by biopsy. Biopsy for rejection diagnosis may have been avoided based on dd-cfDNA result.

DART observational patient. Donor: 1.5y/o Caucasian male, KDPI 79%, CMV D+/R-. Recipient 47y/o African American male with ESRD due to hypertension. PRA 0%, induction with basiliximab at 20mg IV x 2 doses. CMV prophylaxis with valganciclovir 900mg/day. Maintenance IS: tacrolimus, MMF, prednisone.



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