Donor-derived Cell-free DNA Improves DSA-informed Diagnosis of ABMR in Kidney Transplant Patients

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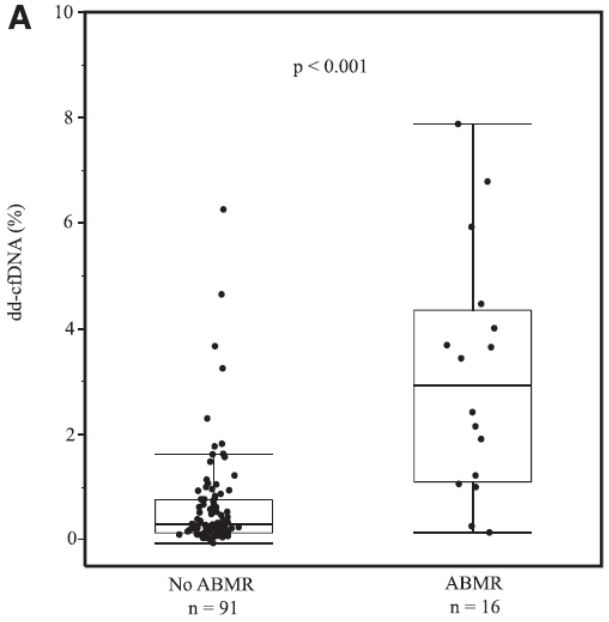
Background

- Antibody mediated rejection (ABMR) is an important cause of acute and chronic allograft dysfunction and graft loss
- Preformed or *de novo* donor-specific antibodies (DSAs) may lead to ABMR, but are not diagnostic alone
- Donor-derived cell-free DNA (dd-cfDNA) is a measure of active injury in DSA positive transplant patients, discriminating ABMR from no ABMR

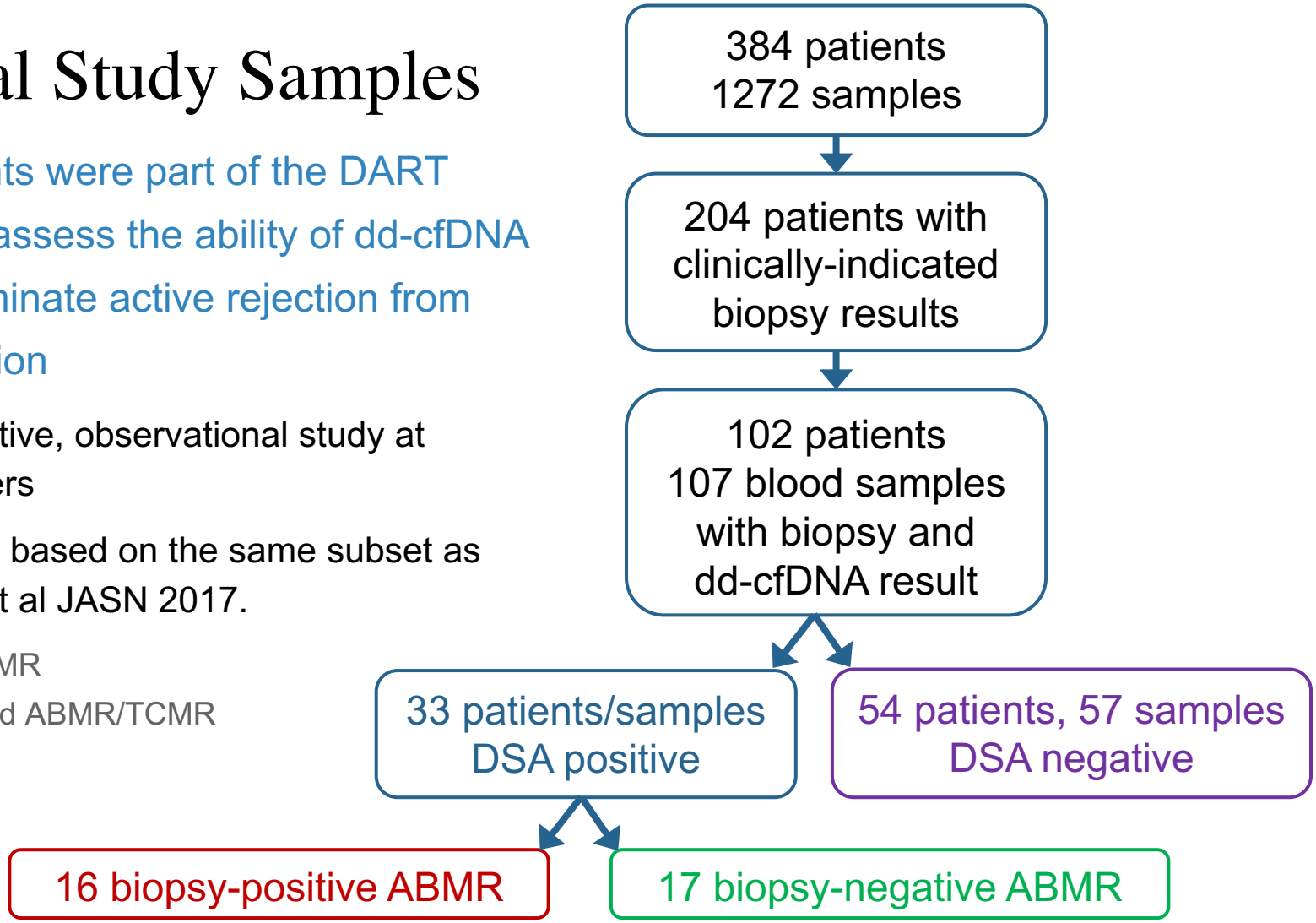


Bloom et al., JASN 2017

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

Clinical Study Samples

- All patients were part of the DART study to assess the ability of dd-cfDNA to discriminate active rejection from no-rejection
  - Prospective, observational study at 14 centers
  - Analysis based on the same subset as Bloom et al JASN 2017.
    - 11 ABMR
    - 5 mixed ABMR/TCMR



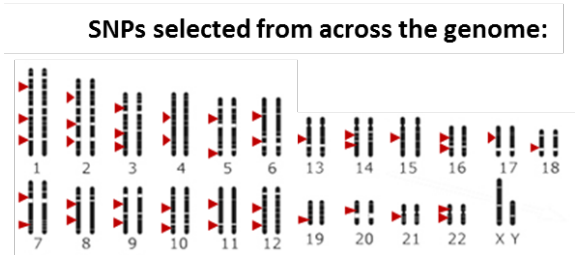
Methods

- DSA positive vs negative determined by center protocol, generally 1000 MFI threshold
- Mixed rejections (ABMR plus TCMR) included in the ABMR group. ABMR defined per Banff 2013
- dd-cfDNA measured using an analytically and clinically validated assay, AlloSure®

  (Grskovic et al., JMD 2016. Bloom et al JASN 2017, Bromberg et al., JALM 2017)
- dd-cfDNA cutoff of 1% used to classify dd-cfDNA as positive or negative

Methods: dd-cfDNA Measurement

- dd-cfDNA is measured by determining the fraction of donor-derived alleles at single-nucleotide polymorphism (SNP) locations
- 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
  - Next-Generation Sequencing is used to count each allele



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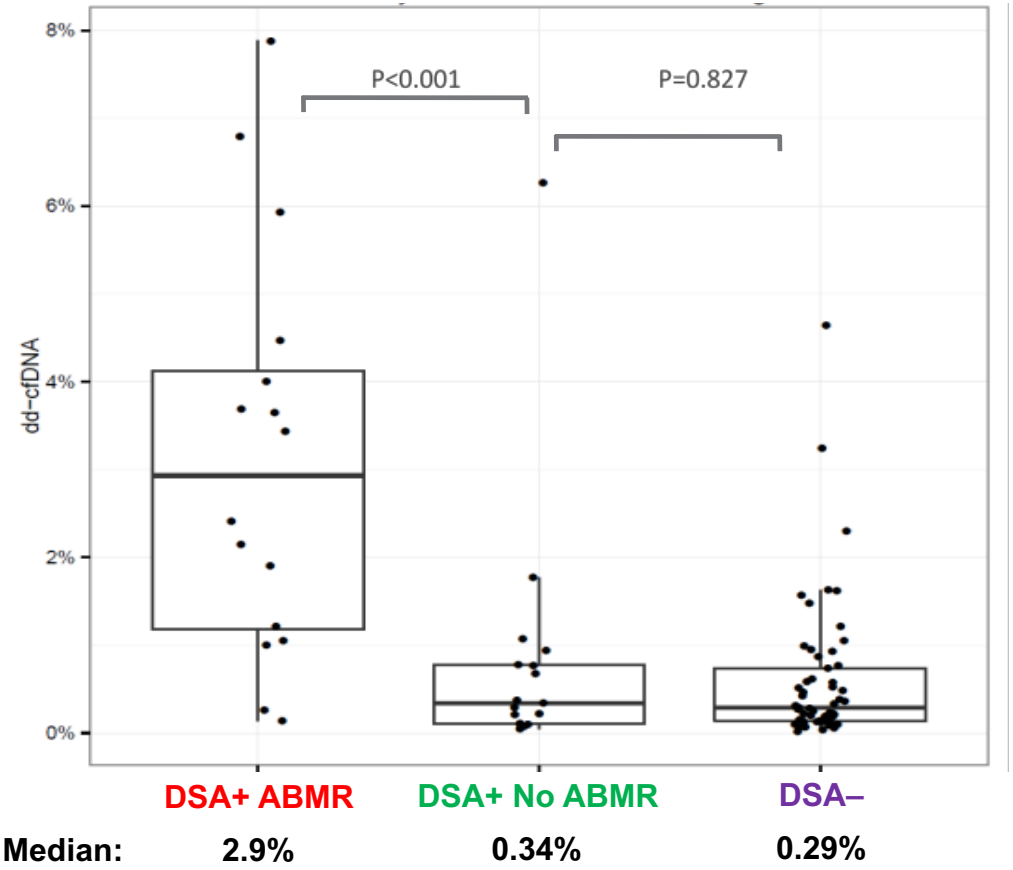
Grskovic M et al. Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. *J Mol Diagn.* 2016;18(6):890-902.

Demographics

	All	DSA+	DSA–	p value
Number	90	33	57	
Age		47 ±14	53 ±13	0.021
Gender (% male)	60%	58%	61%	0.824
Race (% Caucasian)	51%	48%	53%	0.542
Donor Type				0.259
Deceased	61%	67%	58%	
Living unrelated	26%	15%	32%	
Living related	13%	18%	10%	

Results: dd-cfDNA is Higher in DSA+ Patients With Biopsy-Diagnosed ABMR



Results: Combined DSA and dd-cfDNA More Reliably Detects ABMR Than Either Alone

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 for ABMR in DART

(confidence intervals shown in parentheses)

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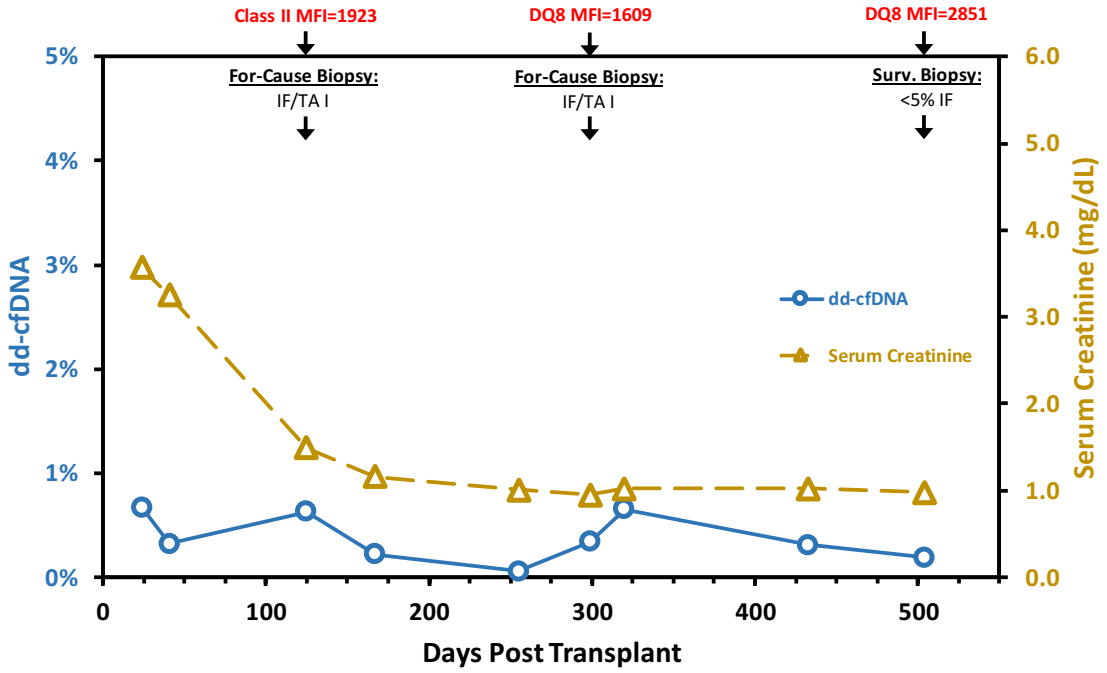
Case Study: DSA Positive Without Evidence of Active Injury by dd-cfDNA

- 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 basiliximab 20mg IV x 2 doses
- CMV: valganciclovir 900mg/day
- Maintenance: tacrolimus, MMF, prednisone



Implications:

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

Limitations:

- Replication and larger studies will enhance the understanding of dd-cfDNA in ABMR
- Comparator biopsy pathology interpretation is subject to inter-reader variability
- A more detailed stratification of the DSA positives (e.g. MFI levels, HLA class type, C1q status, etc) may be informative

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.