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BACKGROUND

- The standard test to differentiate rejection and injury in kidney transplants is the allograft biopsy
- Donor-derived cell-free DNA (dd-cfDNA) is a noninvasive test of allograft cell injury that may enable more frequent, quantitative and safer assessment of allograft status¹⁻²

OBJECTIVES

- The Circulating Donor-Derived Cell-Free DNA in blood for diagnosing Acute Rejection in Kidney Transplant Recipients (DART) study was designed to validate that plasma levels of dd-cfDNA can discriminate between active rejection status and stable transplant
- The reference range of dd-cfDNA in clinically stable renal transplant patients was also characterized

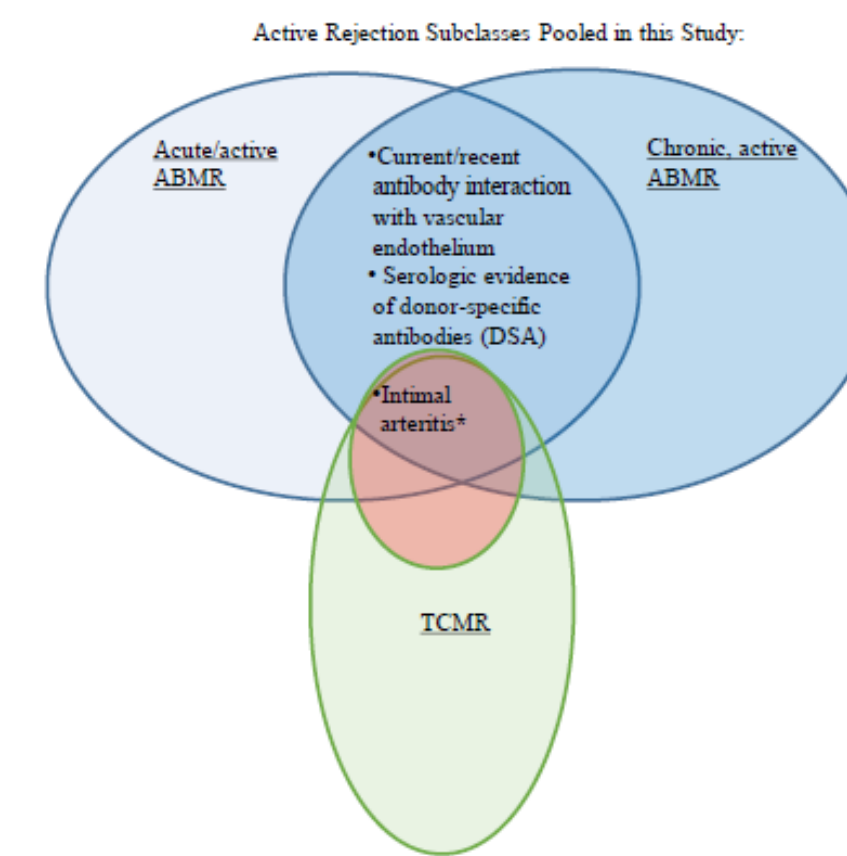
METHODS

- Blood specimens were prospectively collected from kidney recipients at scheduled post-transplant intervals, as well as concomitantly with clinically indicated or protocol kidney biopsies
- dd-cfDNA levels were measured in blood plasma and correlated with allograft rejection status ascertained by renal biopsy
- Independent review of the pathologists' reports to confirm that the findings met the criteria as defined in the Banff Working Group classification system
- dd-cfDNA was measured using a validated clinical-grade targeted NGS method³

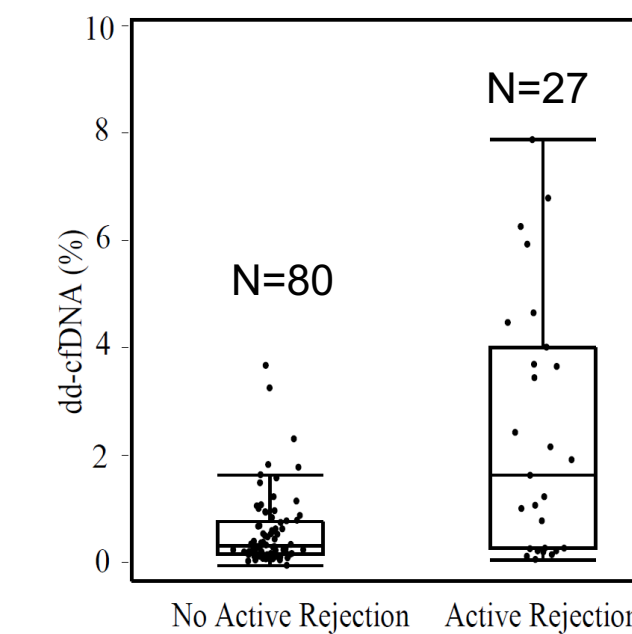
RESULTS

- Of 384 patients with 1272 samples, 219 patients had at least one renal biopsy
- Main study cohort consists of 102 patients with 107 paired biopsies and blood samples, 27 patients in the rejection group and 75 in the no-rejection group

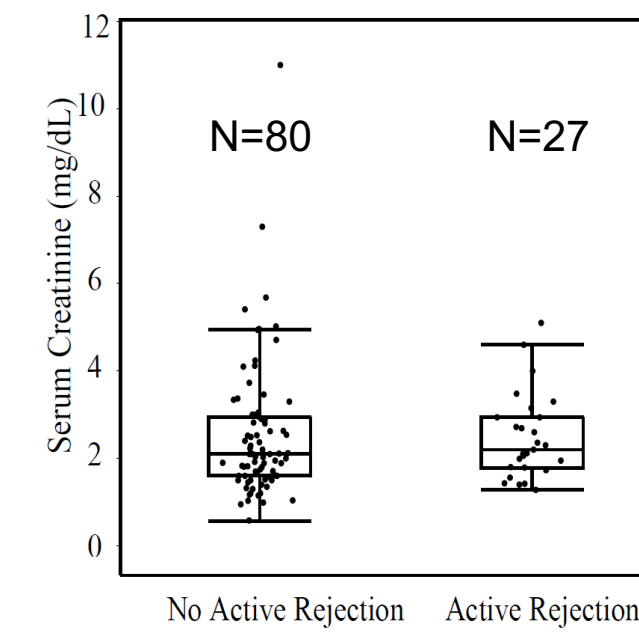
Three histological rejection classes were combined to define "active rejection" based on common injury features



dd-cfDNA discriminates Active Rejection from No Active Rejection

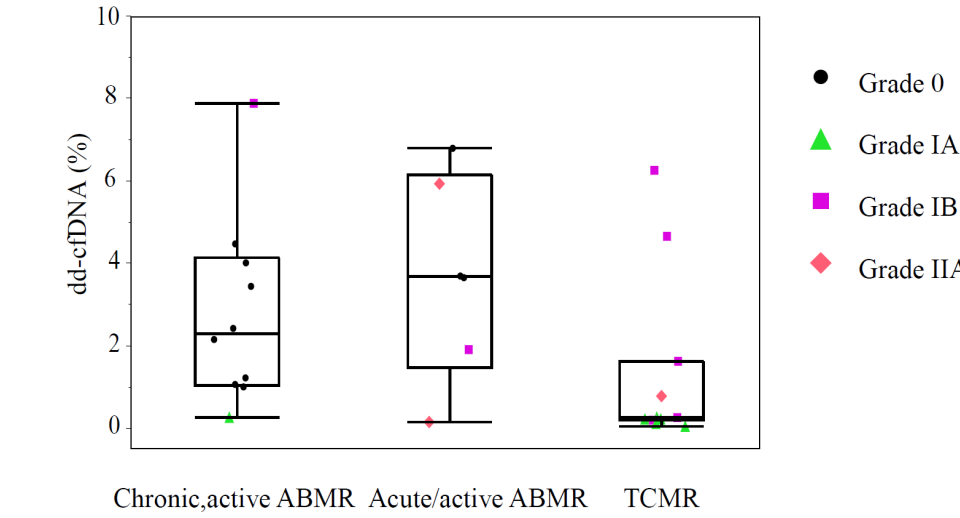


Serum Creatinine does not discriminate Active Rejection from No Active Rejection



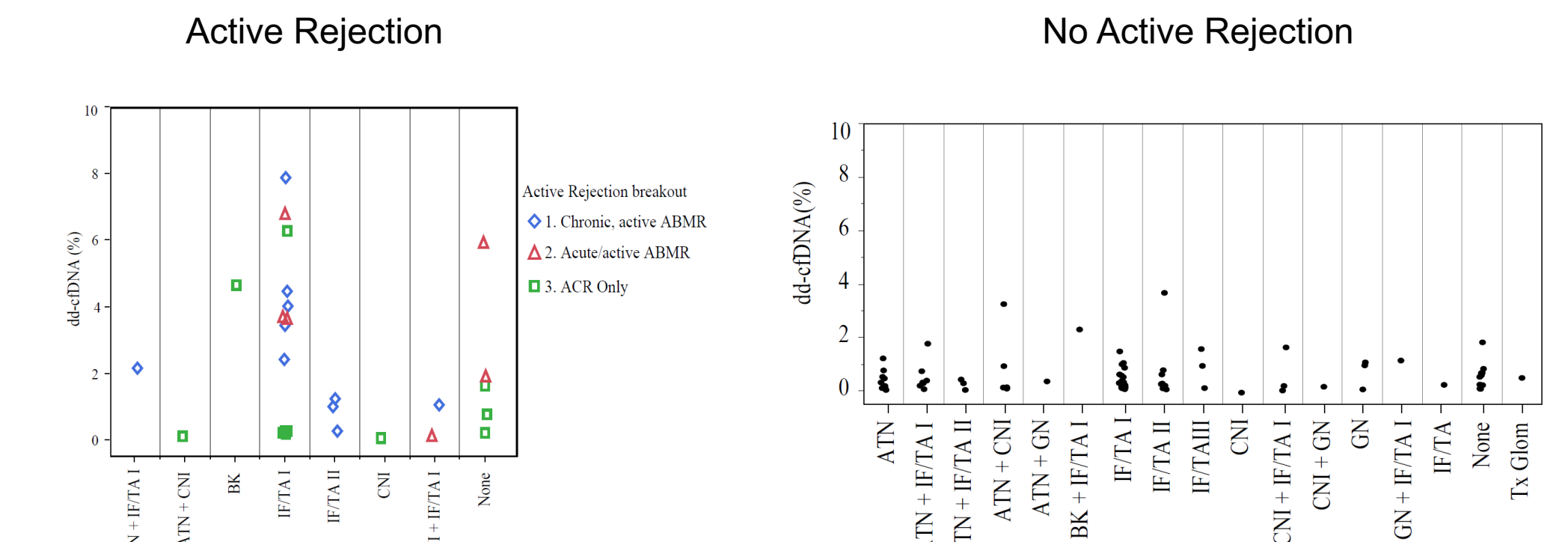
Left: Median dd-cfDNA in active rejection 1.6% versus 0.3% for no rejection (p<0.001). Right: Serum creatinine was not significantly different in median values between two groups (p=0.23).

dd-cfDNA is Higher in ABMR than in T Cell Mediated Rejection



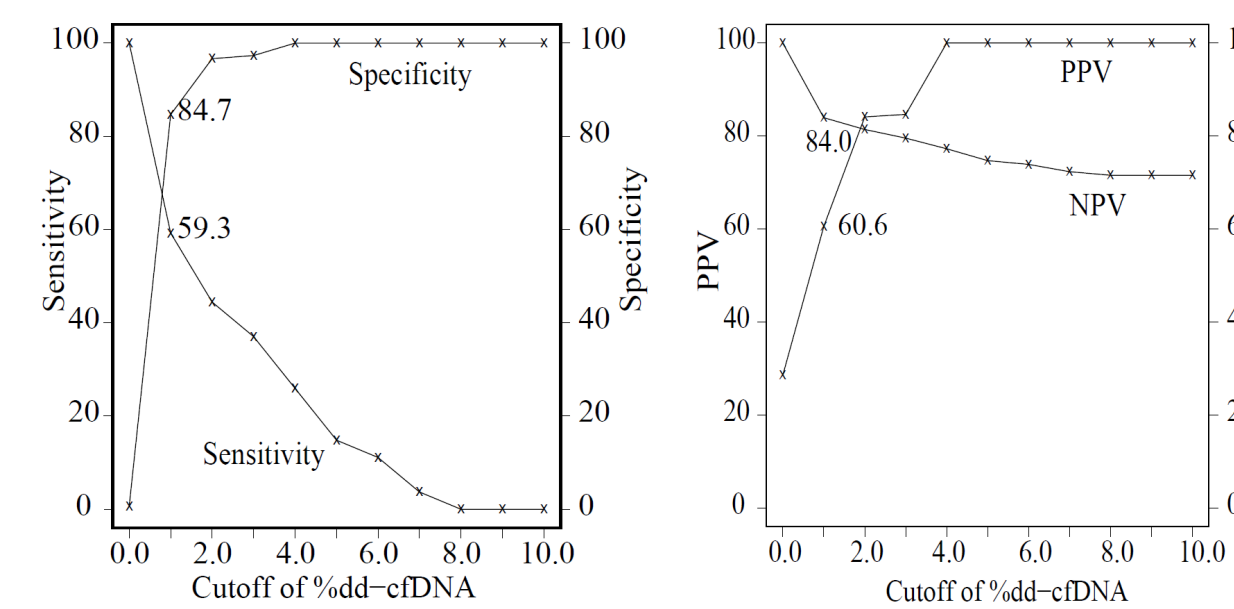
Results of clinically indicated biopsies by rejection subclass. dd-cfDNA in 27 biopsy-based rejections: 10 chronic, active ABMR; 6 acute/active ABMR; 11 TCMR, Types IA (5), IB (5) and IIA (1). ABMR without TCMR is shown as a circle (•). Median dd-cfDNA 2.9% (ABMR), 1.2% (TCMR, Types ≥ IB), 0.2% (TCMR Type IA).

Results of clinically indicated biopsies categorized by non-rejection histologic findings



dd-cfDNA Discriminates Active Rejection

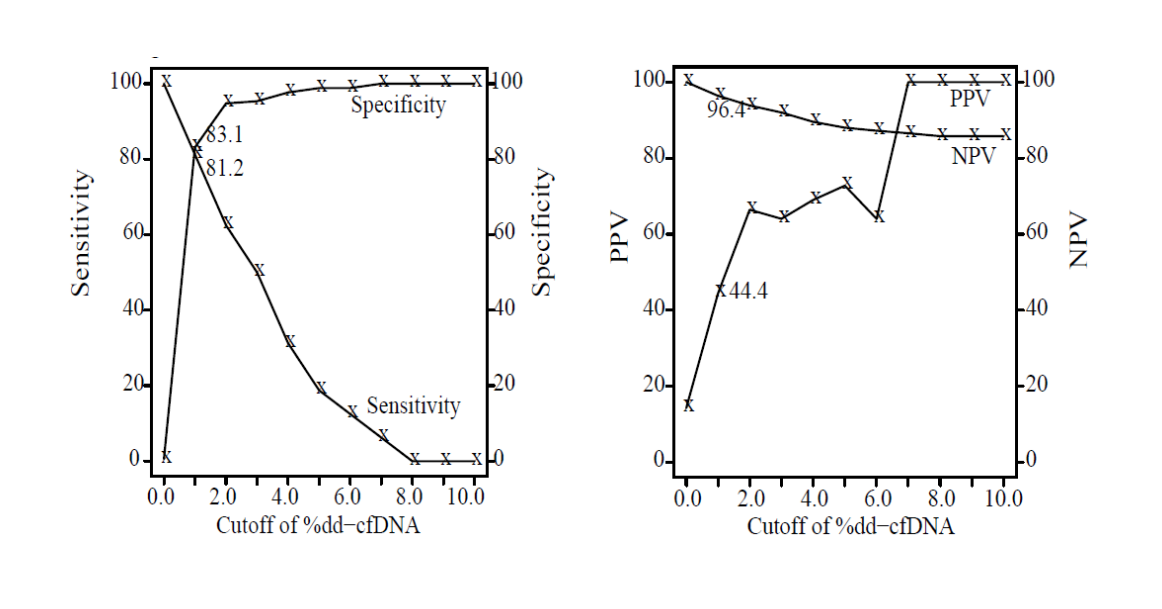
The area under the curve - ROC is 0.74 (95% CI, 0.61- 0.86)



Left: The sensitivity (%) and specificity (%) for dd-cfDNA to discriminate active rejection. Right: The positive predictive value (PPV) and negative predictive value (NPV).

dd-cfDNA Discriminates Antibody-Mediated Rejection

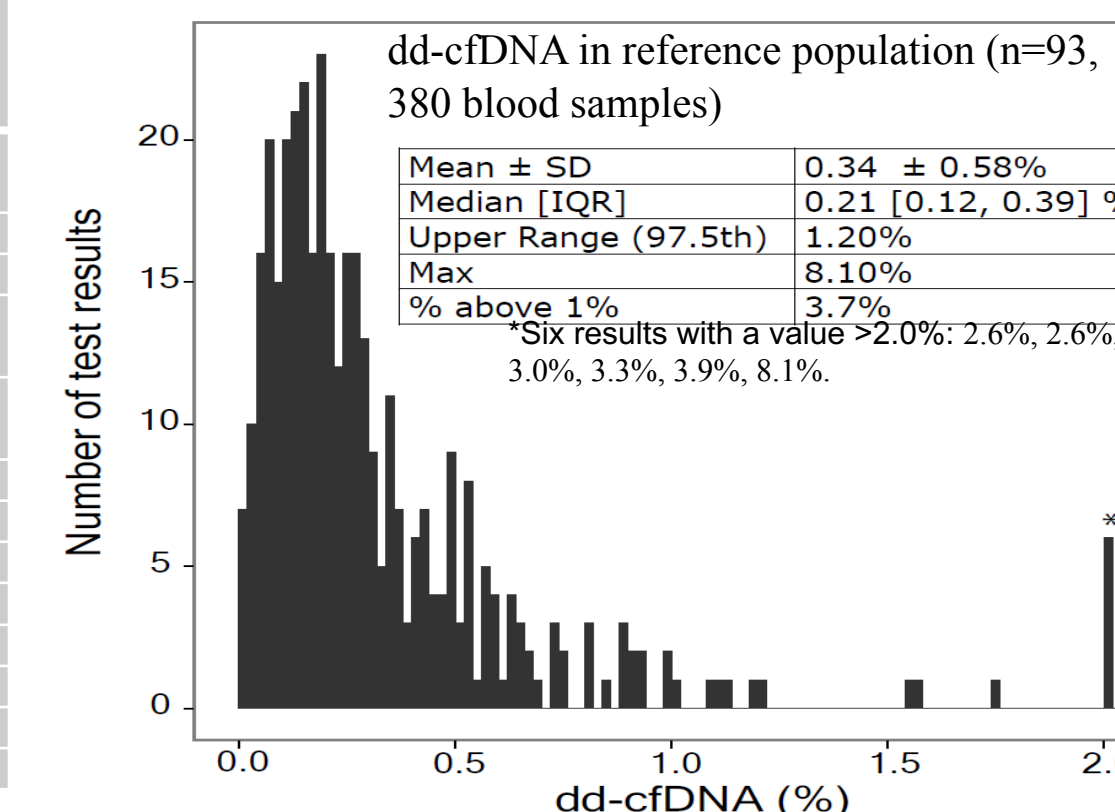
The area under the curve - ROC is 0.87 (95% CI, 0.75- 0.97)



Left: The sensitivity (%) and specificity (%) for dd-cfDNA to discriminate active ABMR. Right: The positive predictive value (PPV) and negative predictive value (NPV).

Renal allograft biopsies and histology-based diagnoses of rejection

	For-Cause		Surveillance		Rejection Follow-Up		Total	
	Patients	Biopsies	Patients	Biopsies	Patients	Biopsies	Patients	Biopsies
Active Rejection	52	58	1	1	1	2	53	61
ABMR or mixed	27	29	0	0	0	0	27	29
Chronic, active ABMR	16	17	0	0	0	0	16	17
Acute/active ABMR	12	12	0	0	0	0	12	12
TCMR only	27	29	1	1	1	2	28	32
Type IA	9	9	1	1	0	0	10	10
Type IB	12	13	0	0	1	1	12	14
Type IIA	5	5	0	0	0	0	5	5
Type IIB	0	0	0	0	1	1	1	1
Type IIC	2	2	0	0	0	0	2	2
No Rejection**	123	146	32	33	2	2	149	181
Total	170	204	33	34	3	4	206	242



CONCLUSIONS

- dd-cfDNA level may be used to assess allograft rejection and injury status
- A cutoff of ≥1% indicates a risk of active rejection (most likely ABMR or TCMR types ≥1B)
- dd-cfDNA levels below threshold reflect absence of moderate or greater active rejection and may be useful to guide immunosuppressive management

REFERENCES

- De Vlamincq, et al. *Sci Transl Med*, 6:24ra277, 2014
- De Vlamincq, et al. *Proc Natl Acad Sci U S A*, 112: 13336-13341, 2015
- Grskovic, et al. *J Mol Diagn*, 18: 890-902, 2016