Donor-Derived Cell-Free DNA is a Dynamic Biomarker of Active Rejection in Kidney Allografts

Daniel C. Brennan, MD[1], Jonathan S. Bromberg, MD, PhD[2], Emilio D. Poggio, MD[3], David Hiller, PhD[4], John J. Sninsky, PhD[4], Robert N. Woodward, PhD[4], James P. Yee, MD, PhD[4] and Roy D. Bloom, MD[5]

[1]Washington University, St. Louis, MO, [2]University of Maryland, Baltimore, MD, [3]Cleveland Clinic, Cleveland, OH, [4]CareDx, Brisbane, CA, and [5]University of Pennsylvania, Philadelphia, PA

Institution	Principal Investigator
Cleveland Clinic Foundation	Emilio Poggio, MD
University of Maryland	Jonathan Bromberg, MD PhD
University of Pittsburgh Medical Center	Puneet Sood, MD
University of Pennsylvania Health System	Roy Bloom, MD
University of Minnesota	Arthur Matas, MD
Baylor Research Institute	Bernard Fischbach, MD
Washington University	Daniel Brennan, MD
Cedars-Sinai Medical Center	Stanley Jordan, MD
Vanderbilt University	Anthony Langone, MD
University of California, Los Angeles	Suphamai Bunnapradist, MD
University of Alabama, Birmingham	Shikha Mehta, MD
Indiana University	Asif Sharfuddin, MD
Baylor Scott & White Memorial Hospital	Mohanram Narayanan, MD
Columbia University	David Cohen, MD

Introduction

Donor-derived cell-free DNA (dd-cfDNA) has shown promise as a biomarker for identification of allograft rejection. In kidney transplantation, dd-cfDNA is higher at the time of Active Rejection as compared to No Rejection at the time of a renal biopsy performed for clinical suspicion of rejection (Bloom 2017)[1]. Here we report on the dynamic change of dd-cfDNA levels from the months prior to rejection, at the time of biopsy-proven rejection, and the months following. The impact of rejection treatment on dd-cfDNA levels is examined in rejections biopsy-proven or diagnosed without biopsy. All conditions are also analyzed for association with serum creatinine.

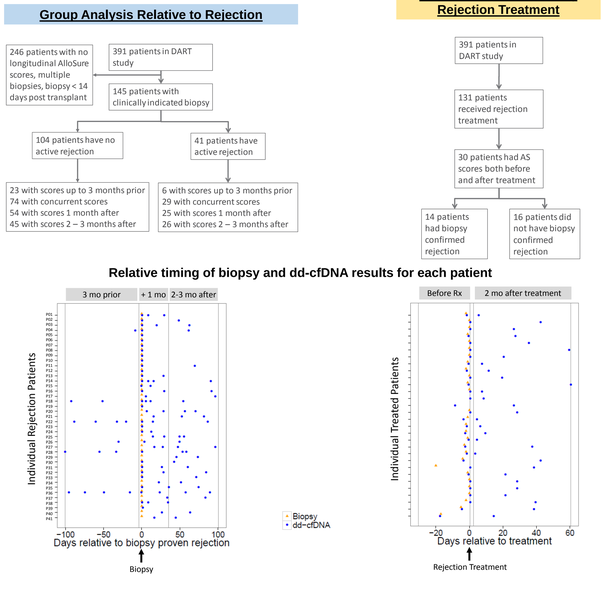
Methods

We measured dd-cfDNA in serial blood specimens from kidney recipients that had a clinically indicated biopsy. 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). dd-cfDNA was quantified in the CareDx CLIA laboratory using a clinical-grade targeted next generation sequencing method (AlloSure, Grskovic 2016)[2].

Two sets of analysis were performed. A <u>group analysis</u> examined dd-cfDNA levels before and after biopsy-proven rejection without requirement for serial samples within a given patient. Banff criteria for T-cell mediated rejection (TCMR) or antibody-mediated rejection (ABMR) were reported in 41 biopsies. Of these, 29 biopsies had a dd-cfDNA sample concurrent with biopsy, 6 patients with biopsy-proven rejection had 14 samples in the three months before biopsy, 25 patients with biopsy-proven rejection had 32 samples in the first month following biopsy, and 26 patients with biopsy-proven rejection had 33 samples in months 2 and 3 following biopsy. These results are compared to 104 patients with for-cause biopsies that lacked evidence of active rejection (no active rejection).

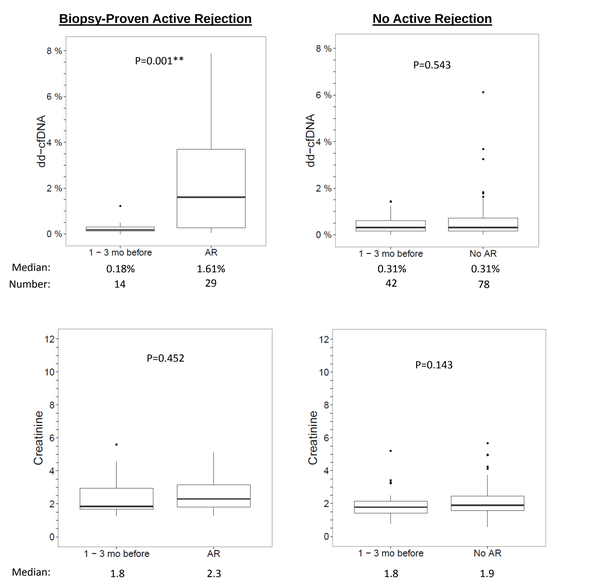
A <u>paired analysis</u> examined dd-cfDNA in response to rejection treatment. 131 patients received rejection treatment, of which 81 had a biopsy prior to treatment, and 30 of these had serial dd-cfDNA measurements within 1 month prior to treatment and another within 2 months after treatment.

Non-parametric methods were used for both group and paired analysis. Single asterisks mark P-values <0.05. Double asterisks mark P-values <0.01. A reference group of 380 samples from 93 transplant recipients with stable renal function was used as a control (Bromberg 2017)[3], in which the median dd-cfDNA is 0.21%.



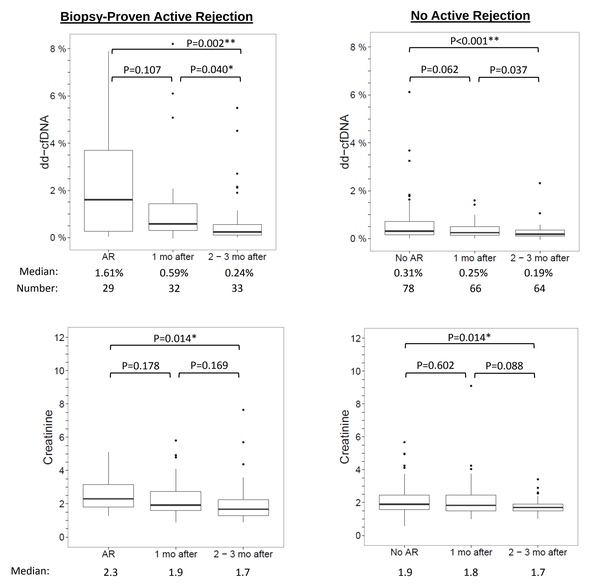
Results

Figure 1. Group analysis shows dd-cfDNA is higher at rejection than prior to active rejection. Serum creatinine does not differ.



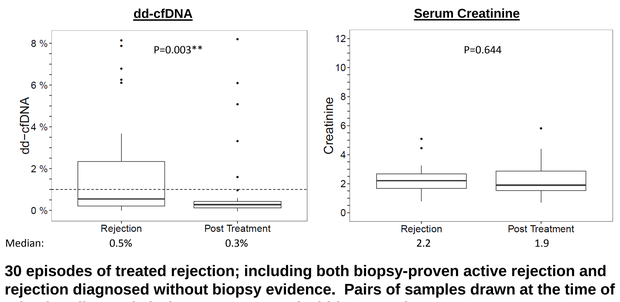
Group analysis. Not all patients have samples in both groups. Wilcoxon analysis to determine significance of the difference in dd-cfDNA levels between times prior to biopsy and concurrent with biopsy. Left panels are biopsy proven active rejection (TCMR or ABMR), right panels negative for active rejection pathology. Top panels dd-cfDNA, bottom panels serum creatinine (mg/dL)

Figure 2. Group analysis shows that dd-cfDNA is lower in the three months following active rejection.



Group analysis. Not all patients have samples in all three groups. Wilcoxon analysis to determine significance of the difference among times concurrent with biopsy and the first month or second to third month following the biopsy. Left panels are biopsy proven active rejection (TCMR or ABMR), right panels negative for active rejection pathology. Top, dd-cfDNA, bottom, serum creatinine (mg/dL).

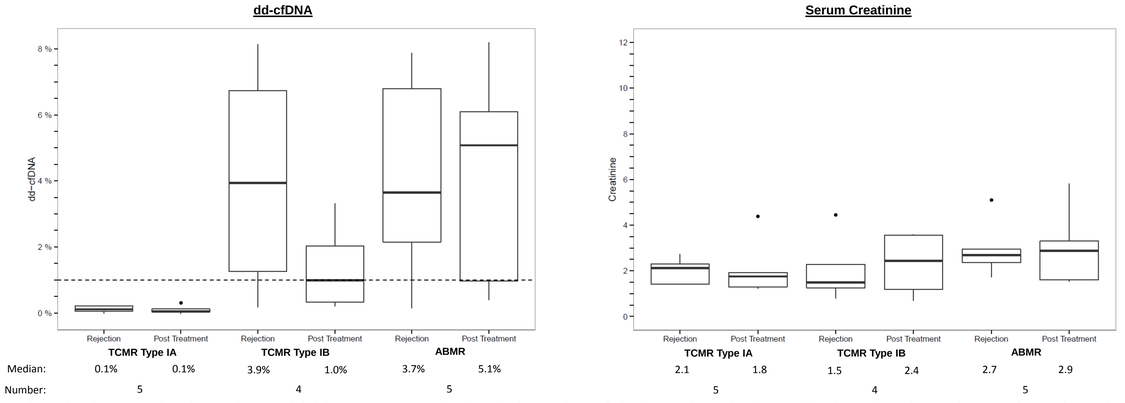
Figure 3. Paired analysis demonstrates that dd-cfDNA is reduced by rejection treatment but serum creatinine does not change.



30 episodes of treated rejection; including both biopsy-proven active rejection and rejection diagnosed without biopsy evidence. Pairs of samples drawn at the time of <u>rejection</u> diagnosis before treatment and within 2 months <u>post treatment</u>.

For a subset of 12 samples with dd-cfDNA > 1% at rejection, dd-cfDNA is reduced from 3.7% to 0.4%, a median 80% reduction.

Figure 4. Paired analysis: dd-cfDNA is elevated in TCMR IB and ABMR and is reduced following treatment for TCMR.



Paired data by rejection type for the patients with biopsy-proven rejection (subset of 14 of the 30 patients in Figure 3). The pairs of samples are shown in each plot, the first bar showing the data from the time of biopsy-proven rejection and the second bar showing data from the same patients within 2 months post treatment. Treatments included: IA, 3 pulse Solumedrol, 1 pulse Solumedrol and prednisone, 1 ATG; IB, pulse Solumedrol or prednisone; ABMR, all pulse Solumedrol and plasmapheresis, 2 IVIG and Rituximab, 1 IVIG, and 1 Rituximab and ATG.

Conclusions

- **dd-cfDNA is a dynamic biomarker that rises at the time of biopsy-defined rejection and is reduced following rejection. Serum creatinine does not display the same dynamic signal.**
- **Rejection treatment significantly decreases dd-cfDNA.**
- **Serum creatinine is not significantly changed following rejection treatment.**
- **Confirmed TCMR IB responds to rejection treatment with reduced dd-cfDNA. ABMR does not respond as well to treatment.**
- **As a dynamic biomarker, longitudinal surveillance with dd-cfDNA may be useful to detect and subsequently assess patient recovery from active rejection. Limited response in ABMR may reflect sub-optimal treatment.**

References

[1]Bloom et al., 2017. *Cell-Free DNA and Active Rejection in Kidney Allografts.* J Am Soc Nephrol.

[2]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.

[3]Bromberg et al., 2017. *Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications.* J Applied Lab Medicine.

Disclosures

The DART study was supported by CareDx.
DM, JB, and RB have received grant support from CareDx.
EP has received travel support as a Medical Advisor from CareDx.
RB has received consulting from CareDx.
DH, JS, RW, and JY are employees of CareDx.