



Donor-Derived Cell-Free DNA Normal Range and Biological Variation Defined in a Reference Population



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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 status¹⁻³

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 active rejection status and to characterize reference ranges for dd-cfDNA stable transplant recipients.
- By establishing clinical reference ranges of dd-cfDNA in stable renal transplant recipients and identifying normal biological variation in serial testing, clinical interpretation of dd-cfDNA results can be enhanced.

METHODS

- Blood specimens were prospectively collected from kidney recipients at scheduled post-transplant intervals (1,2,3,4 and 6 months).
- Patients with stable allograft function across at least 3 serial visits >14 days post transplant were selected as the reference population.
- Reference Population Selection Criteria

Inclusion	Exclusion
Well Functioning grafts	Increase in Serum Creatinine ≥ 0.5 mg/dl Since Prior Testing
No Clinical Suspicion of Rejection	Delayed Graft Function Defined by Ongoing Need for Dialysis in the Post-Transplant Period
Stable and Acceptable Serum Creatinine Values	Clinically Indicated Visit or Renal Transplant Biopsy for Allograft Dysfunction
No Significant Proteinuria	Allograft Acute Rejection Event
No Infections	Active Urinary tract, Cytomegalovirus (CMV) or Polyomavirus Type BK (BKV) infections.
No Acute Cardiovascular Changes or Other Acute Clinical Event	Prior Organ Transplant Remained <i>in situ</i>

- dd-cfDNA was measured using a validated clinical-grade targeted NGS method with an established analytical coefficient of variation (CV) of 6.8%.⁴
- Reference change values (RCV) and Coefficient of Variations (CV) computed using standard formulas.⁵

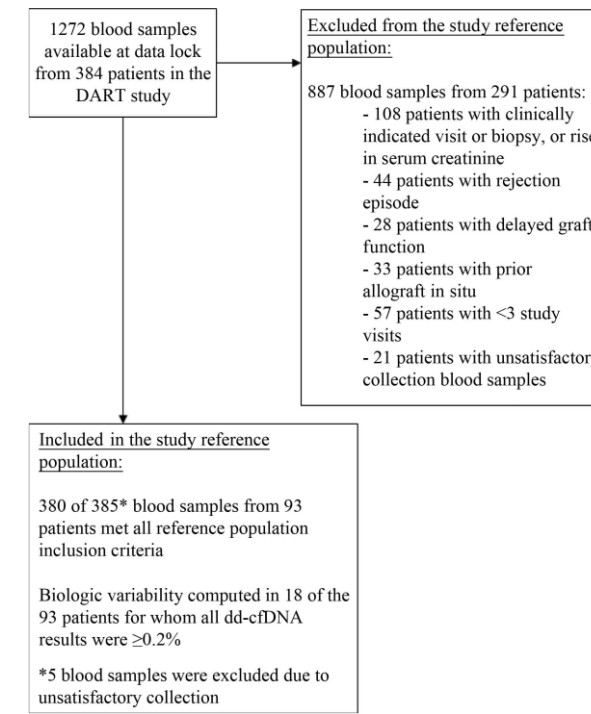
RESULTS⁶

DART Study Sites and Principal Investigators

Institution Name	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

Of 384 patients with 1272 samples, 93 patients met the inclusion/exclusion criteria for reference population with ≥3 sample for a total of 380 samples that met dd-cfDNA test and sample QC

Biologic variability computed in 18 of 93 patients where dd-cfDNA ≥0.2% (limit of detection)



Clinical Characteristic	DDC [†] Exclusion	DDC [†] Inclusion	DDC [†] Exclusion	DDC [†] Inclusion	p-value [‡]	Renal Transplant Failure at 1 Y [§]
Number of Patients	384	384	291	380		12.8%
Number of Samples	1272	1272	887	1272		5%
Number of Sites	11	11	11	11	0.53	30%
Age	51.1	51.1	51.1	51.1	0.99	12.8%
Black or African American	107 (28%)	107 (28%)	107 (28%)	107 (28%)	0.99	12.8%
Hispanic	11 (3%)	11 (3%)	11 (3%)	11 (3%)	0.99	12.8%
Other Race or Ethnicity	166 (43%)	166 (43%)	166 (43%)	166 (43%)	0.99	12.8%
Time to Transplant	1.2	1.2	1.2	1.2	0.99	12.8%
Time to Biopsy	1.2	1.2	1.2	1.2	0.99	12.8%
Time to Death	1.2	1.2	1.2	1.2	0.99	12.8%
Time to Rejection	1.2	1.2	1.2	1.2	0.99	12.8%
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