Clinically Relevant Variation of Donor-Derived Cell-Free DNA During Longitudinal Surveillance of Renal Allografts

Mohanram Narayanan[1], Tarek Alhamad[2], David Hiller[3], Jim Yee[3], Robert N Woodward[3], Jonathan S Bromberg[4]

[1]Baylor Scott &White, [2]Washington University, [3]CareDx, [4]University of Maryland

INTRODUCTION

Donor-derived cell free DNA (dd-cfDNA) has been identified as a biomarker for rejection in kidney transplantation[1]. dd-cfDNA levels ≥ 1% are associated with active rejection in kidney allografts. An assay for dd-cfDNA (AlloSure®) has been analytically validated[2] and reference values have been characterized in a cohort of patients from the DART study with stable allograft function over at least 3 serial visits[3].

In this current analysis, dd-cfDNA is characterized in a more broad cohort: patients in DART who did not have rejection and who had up to 11 tests at surveillance visits during the first 2 years post-transplantation. Within and between patient variability is computed and clinically relevant variation of dd-cfDNA is characterized.

Table 1. Surveillance cohort is broader than reference cohort

Inclusion Criteria	Surveillance cohort	Reference cohort[3]
Time of first visit	≤ 75 days post transplant	Anytime
Delayed graft function patients	Yes	No
Patients with creatinine changes (delta ≥ 0.5)	Yes	No
Patients with rejections	No	No
Patients with infections	Yes	No
Patients with clinically indicated biopsies or visits?	Yes, unless first visit was clinically indicated	No
Min number of visits per patient	1	3

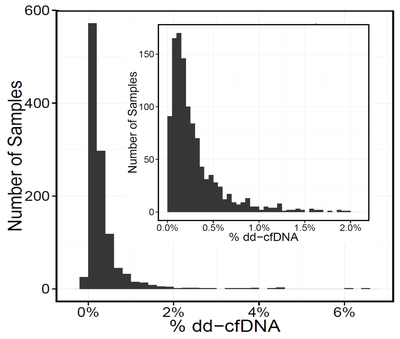
METHODS

Inclusion criteria for the surveillance cohort are listed in Table 1 and compared to the inclusion criteria for the reference cohort[3]. The reference cohort was a "pristine" patient cohort (with no significant medical issues such as DGF or infection, and without clinical signs of rejection), whereas the surveillance cohort reflects the biological variability across all patients without rejection. Samples collected were between 2 weeks and 2 years post-transplantation

Of 347 patients in DART, 202 patients with 1152 tests met the requirements for the surveillance cohort. Normal ranges of dd-cfDNA in this cohort were calculated as were within (CV_I) and between (CV_G) patient variability, index of individuality (II) and reference change value (RCV); these were compared to the same values in the reference cohort. II is the ratio of within to between patient variability; II > 0.60 suggests that a biomarker has similar distribution across patients[4]. RCV is the observed change in serial measurements that exceeds biological variability.

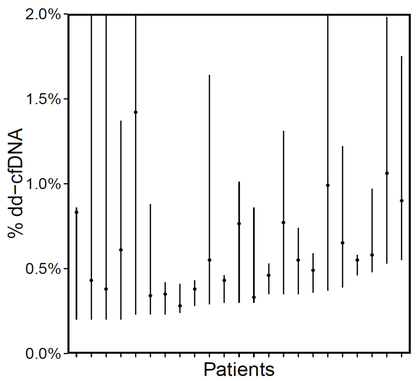
Outlier samples with dd-cfDNA ≥ 1% were counted and patients with multiple outliers examined more closely. Average dd-cfDNA results by month over the first 2 years post transplantation were examined for trends.

Figure 1: dd-cfDNA was very low in the surveillance cohort. Median dd-cfDNA was 0.19% with interquartile range 0.10% – 0.35%. Over half the samples were below 0.2%, the lower limit of detection. Plot of distribution (left). Summary statistics of surveillance cohort compared to reference cohort (right). Surveillance cohort is twice as large with an average of two more samples per patient; distribution of dd-cfDNA in the surveillance cohort is similar to distribution in the reference cohort except the surveillance cohort contains more values ≥ 1%.



	Surveillance Cohort	Reference Cohort[3]
Number of patients	202	93
Number of samples	1152	380
Samples / patient	5.7	4.1
Mean ± SD	0.34% ± 0.54%	0.34% ± 0.58%
Median [IQR]	0.19% [0.10%, 0.35 %]	0.21% [0.12%, 0.39%]
Upper range (97.5th percentile)	1.56%	1.20%
Maximum	6.56%	8.10%
Fraction values above 1%	5.5%	3.9%

Figure 2: dd-cfDNA serial increase of 86% exceeds biological variability; Within patient variability is lower than between patient variability. Patients with at least 3 longitudinal samples with dd-cfDNA levels greater than 0.2%, the lower limit of detection of the test, are used to compute variability statistics. Minimum, median and maximum dd-cfDNA of the 23 patients (left); Within and between patient variability, II, RCV of these 23 patients (right). The RCV (86%) was exceeded in 6% of 495 adjacent tests. Compared to the reference cohort, within and between patient variability, II and RCV were higher in the more diverse surveillance cohort.

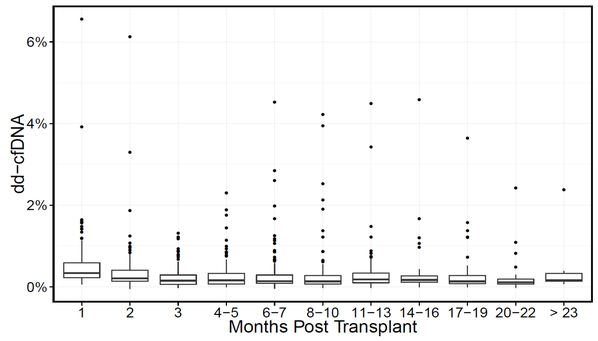


	Surv. Cohort	Ref. Cohort[3]
Number of patients	23	18
Number of samples	115	68
Samples / patient	5.0	3.8
Analytical (CV_A)	6.8%[2]	6.8%[2]
Within- patient (CV_I)	30%	21%
Between-patient (CV_G)	46%	37%
Index of Individuality (II)	0.66	0.57
Reference Change Value (RCV)	86%	61%

Table 2. Most (80%) surveillance patients have no dd-cfDNA values greater than 1% ; in 12 of 14 patients that had two or more high values, the values were on consecutive visits and may have been due to the same ongoing injury.

Number of results ≥ 1% in a patient	Number of patients
0	162 (80%)
1	26 (13%)
2	8 (4%)
3 or more	6 (3%)

Figure 3. dd-cfDNA levels are slightly elevated from 2 weeks to 2 months post transplant and stable from 2 months to 2 years. Boxplot showing levels of dd-cfDNA by month post transplant (top), comparison of median dd-cfDNA levels between 2 weeks to 2 months and levels between 2 months to 2 years using Wilcoxon Rank Sum test (bottom). The fraction of values ≥ 1% was not significantly higher between the two groups.



	2 weeks – 2 months	2 months –2 years
N (no. of patients)	198	191
# Samples (samp / pat)	356 (1.8)	796 (4.2)
Mean ± SD	0.42% ± 0.59%	0.29% ± 0.50%
Median [IQR]	0.29% [0.17%, 0.51%]	0.15% [0.08%, 0.29%]
Upper range (97.5th percentile)	1.49%	1.58%
Maximum	6.56%	4.58%
Fraction values above 1%	6.2%	5.2%
p (difference in dd-cfDNA level)	< 0.001	

CONCLUSIONS

- The reference ranges in this report may help guide the clinical interpretation of a single dd-cfDNA result.
- Because the index of individuality is ≥ 0.6, dd-cfDNA values can confidently be compared across patients using a common threshold of 1%
- An RCV increase in dd-cfDNA of > 86% may be clinically relevant even if the values are below the threshold of 1% previously established for probability of active rejection or other injury.
- Since this cohort of apparently stable renal allograft recipients showed ~20% incidence of unexplained dd-cfDNA levels ≥ 1%, it may be reasonable to repeat testing in a patient who has no other signs or symptoms of allograft dysfunction or injury other than a first observation of a dd-cfDNA score greater than or equal to 1 %.

REFERENCES

1. Bloom RD, et al. Cell-Free DNA and Active Rejection in Kidney Allografts. *Journal of the American Society of Nephrology.* 2017;28(7): 2221-2232.

2. 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.

3. Bromberg JS, et al. Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications. *J Appl Lab Med* 2017.

4. Wu AH. *Biological and analytical variation of clinical biomarker testing: implications for biomarker-guided therapy.* Curr Heart Fail Rep 2013;10:434–440.

Disclosures.
The DART study was supported by CareDx.
MN and JSB: Grant/Research Support CareDx, Honoraria CareDx
TA: Grant/Research Support CareDx
DH, JY, RNW: Employed by CareDx