Donor-Derived Cell Free DNA: Is It All the Same?

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Abstract

Background Clinical utility of donor-derived, cellfree DNA (dd-cfDNA) in transplantation has been extensively reviewed, supporting its use as a surveillance tool for the early and accurate detection of allograft injury. Yet studies comparing different assay methods have been lacking.

Methods Paired sampling of commercially available dd-cfDNA (AlloSure and Prospera) was compared and examined against histology and manufacturer guidance. A total of 76 patients were prospectively assessed, with 11 biopsy sample–proven rejections (antibody-mediated rejection, $n=2$; T cell–mediated rejection, $n=9$).

Results Prospera demonstrated larger measurements of dd-cfDNA in comparison with AlloSure, but this was NS ($P=0.12$). At current manufacturer recommended diagnostic cutoffs, there was no significant difference in sensitivity, specificity, negative predictive value, or positive predictive value of AlloSure versus Prospera in detecting rejection. AlloSure demonstrated a significantly shorter turnaround time ($P=0.01$) from blood draw to patient result.

Conclusions Although dd-cfDNAs are similar, they are not the same. Extensive evidence for dd-cfDNA interpretation remains the key to building clinical utility when considering clinical implementation, and remaining consistent to a single platform is important when creating data comparisons.

Introduction

The clinical utility of donor-derived, cellfree DNA (dd-cfDNA) in transplantation has been demonstrated in the management of patients where it is has been widely used as a surveillance tool for the early and accurate detection of allograft injury. The validity and utility of this important new biomarker has been extensively reviewed (1–3). However, studies comparing different assay methods have been lacking. Therefore, we compared commercially available test services to assess whether any differences are clinically meaningful.

Two tests commercially available for patient management were evaluated: AlloSure (CareDx, Inc., Brisbane, CA) (4,5) and Prospera (Natera, Inc., San Carlos, CA) (6,7). Both have published analytic validation studies but have different degrees of clinical validation. AlloSure has large prospective, multicenter data, whereas Prospera was substantiated using a single-center, retrospective biobank. Both techniques use proprietary library preparation methods and standard next-generation sequencing (NGS) to quantify dd-cfDNA.

NGS technologies enable sequencing of small amounts of DNA in a more quantifiable and robust manner, with acceptable time to return diagnostic tests. For NGS assays to discriminate dd-cfDNA from recipient cell-free DNA, polymorphisms between donor and recipient are exploited. Single-nucleotide polymorphisms (SNPs) are used for building scalable, high-quality tests to quantify dd-cfDNA; however, there is no evidence to show that superior outcomes are associated with the number of SNPs assessed (8).

Current publications around dd-cfDNA are discordant, with some suggesting superiority and use of a specific library preparation technology (6). Yet both AlloSure and Prospera use Illumina-based NGS platforms and SNP methodology, so it remains unclear whether these claims are true or not in the absence of a head-to-head comparison (5,6). The objective of this study is to provide an early real-life, multi-center experience comparing results using the commercially available tests to assess these claims using paired values in renal allograft recipients.

Materials and Methods

Sample Collection and dd-cfDNA Quantification

Between November 2019 and March 2020, 76 single renal transplant patients from our center had simultaneous AlloSure and Prospera dd-cfDNA tests prospectively performed to compare dd-cfDNA levels and report association with clinical outcomes at either George Washington or within the Dallas Nephrology Associate network. All comers were approached for a paired draw. At the time of study, AlloSure was reimbursed by Medicare and being used under a surveillance and for-cause protocol (months 1, 2, 3, 4, 6, 9, and 12 within the first year, and every 3 months

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thereafter), whereas the Prospera test was not reimbursable and used only when paired with an AlloSure kit. Dd-cfDNA samples were collected and associated with surveillance visit or renal biopsy post-transplant (Figure 1). Local institutional review board approval was granted. Interpretation and performance of the tests were based on the manufacturers' guidance and published validity data for the respective assays. Prospera uses the 1% cutoff (7), whereas AlloSure uses a gradient, considering relative change >0.5% (9). Turnaround time (TAT) from blood draw to the returned test result was recorded. Table 1 compares the two assays.

Patient Demographics and Management

Our typical patient populations are highly sensitized, with panel-reactive antibody >20%, a larger black population, more frequent HLA mismatches, and with the primary causes of ESKD caused by hypertension and diabetes (Supplemental Table 1). The paired design with each patient being their own control and having both tests eliminates concern of confounding factors. All patients had thymoglobulin at 3 mg/kg or 1 dose of Simulect at 20 mg. Patients were on extended-release tacrolimus and mycophenolate mofetil for maintenance, with some weaned from steroids early post-operative, while others had donor-specific antibody testing done for cause.

Histologic Reads: Biopsy Specimen–Proven Rejection

Biopsy specimens were read and scored by the local institutional pathologist according to Banff 2017 classification. Results were classified as antibody-mediated rejection ($n=2$), T cell–mediated rejection (TCMR, $n=9$), acute tubular necrosis ($n=4$), BK virus nephropathy (BKVAN; $n=1$), or normal ($n=60$). A total of 52 dd-cfDNA tests were ordered as part of surveillance, with 24 being ordered due to a change in clinical presentation (formation of *de novo* donor-specific antibody, change in creatinine, change in tacrolimus level; Figure 1).

Statistical Analyses

The Kruskal–Wallis test was used to evaluate the distributions of the difference between the paired dd-cfDNA percentages (AlloSure versus Prospera) across the categories of biopsy occurrence. The Wilcoxon signed rank test was used to evaluate the difference between the paired TAT between the assays. The method outlined by Hanley and McNeil (10) was used to derive sample size estimates based upon comparing area under the receiver operating characteristic curve (AUC ROC) for paired data. All statistical tests reported are two-sided, where statistical significance is defined as a P value <0.05.

Results

A total of 76 samples were assessed with both AlloSure and Prospera, which included 11 (14%) biopsy specimen–proven rejections. Measurements of dd-cfDNA between the testing methods for the paired samples (excluding one paired sample with AlloSure score of >16 and Prospera score of 19.61) tend to demonstrate larger measurements for Prospera in comparison with AlloSure, as shown in Figure 2A (0%–1%) and Figure 2B (0%–20%) (Kruskal–Wallis chi-squared test=2.5916, df=2, $P=0.27$). This trend was most apparent for samples with associated biopsy specimen–confirmed rejections (Figure 2C).

Dd-cfDNA as a molecular marker of injury may be elevated by other causes, including BKVAN. The one patient with BKVAN had simian vacuolating virus 40 staining on their biopsy specimen, with blood-based PCR load of 100,000 copies. AlloSure has evidence suggesting the degree
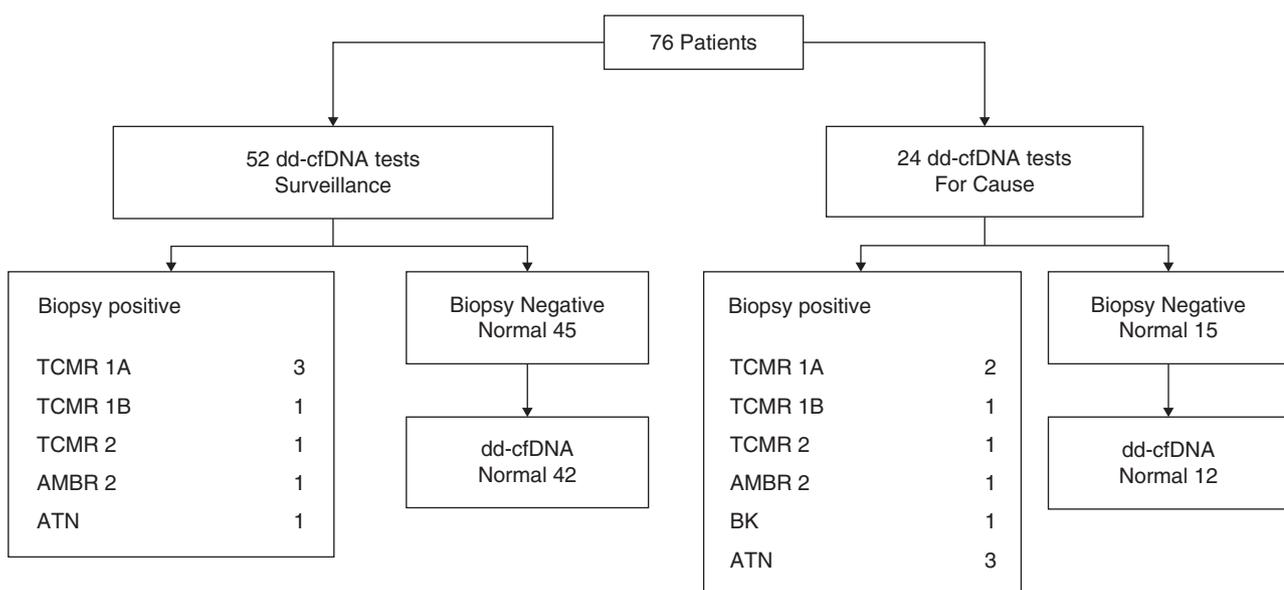

Figure 1. | **Workflow of patients investigated with AlloSure and Prospera under surveillance or for-cause donor-derived, cellfree DNA (dd-cfDNA) testing.** AMBR, antibody-mediated rejection; ATN, acute tubular necrosis; BK, BK virus nephropathy; TCMR, T cell–mediated rejection.

Table 1.   Side-by-side characteristics of AlloSure versus Prospera

Characteristic	AlloSure	Prospera
Limit of detection	0.12%	0.15%
Claimed sensitivity for TCMR	RCV >0.5% (9)	100% at 1% (7)
Claimed AUC for all rejection	77%	87%
Number of SNPs	405	13,962
Precision (CV)	5% and 8% for samples above and below 2% dd-cfDNA, respectively (5)	4% (6)
Lowest input material needed	3 ng (5)	15 ng (6)
Blood tubes needed	1	2

TCMR, T cell–mediated rejection; RCV, relative change value; AUC, area under the curve; SNP, single nucleotide polymorphism; CV, change value; dd-cfDNA, donor derived, cell-free DNA.

of injury can differentiate viremia from nephropathy, but the sample is small with active trials undergoing. Prospera has no data on its utility on BKVAN (11).

No significant differences among diagnostic test characteristics were observed, despite AlloSure trending higher in performance. This is based upon the estimates obtained and their associated 95% confidence intervals (Tables 2, 3, and 4). A prohibitively large number of samples would be required to delineate the nominal differences, which are not clinically relevant. For example, the estimated AUC ROC for AlloSure and Prospera are 0.7343 and 0.7483, respectively. To demonstrate AUC ROC superiority of Prospera relative to AlloSure, assuming a significance level of 5%, power to detect a difference of 80%, correlation among the paired samples of 96%, and 15% of the biopsies performed resulting in rejection; the estimated number of samples that would need to be collected, tested across both diagnostic platforms, and have biopsies performed to confirm rejection is >2300.
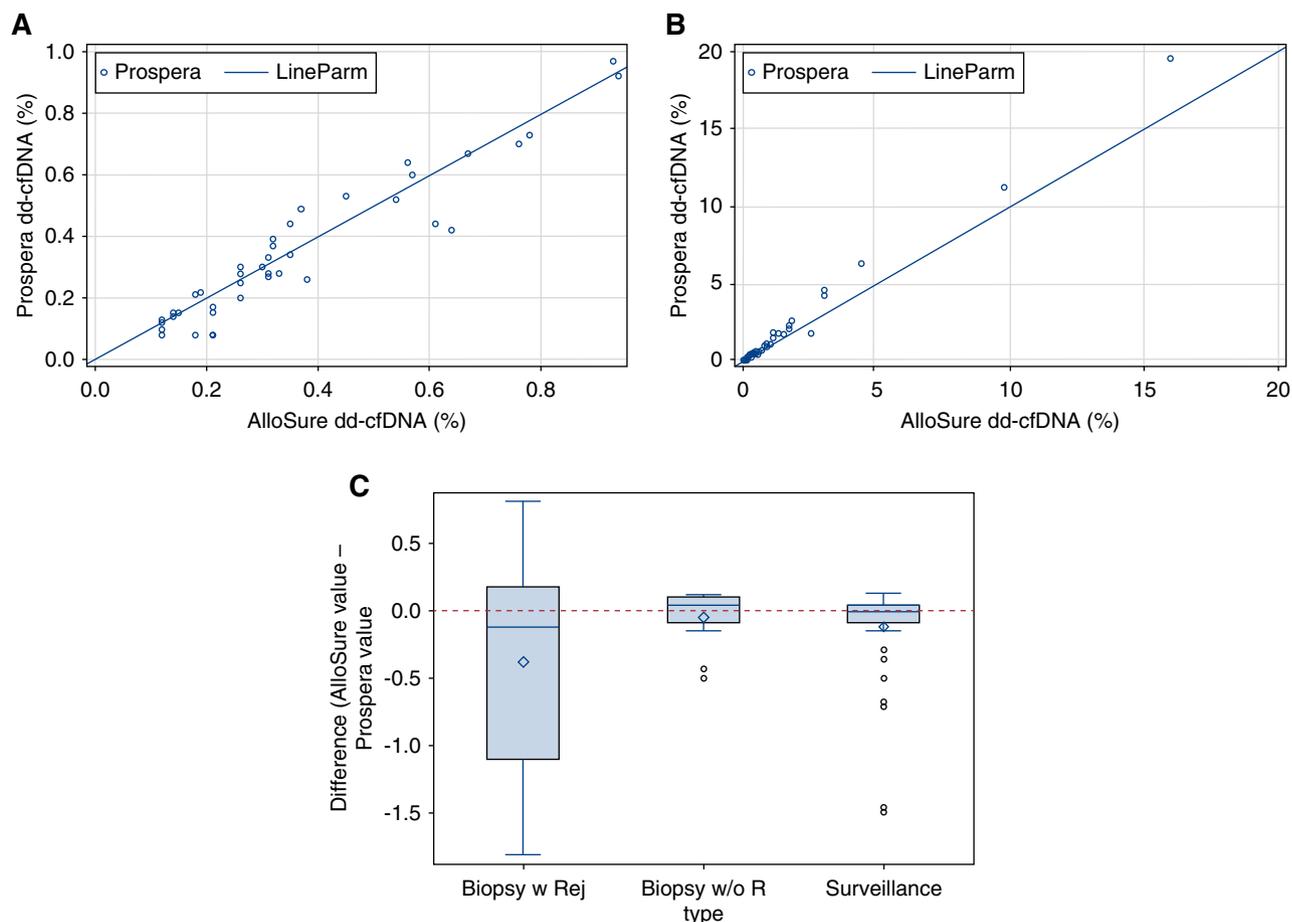

Figure 2. | Three-part panel showing comparison of the difference in dd-cfDNA percentages across all sample-paired dd-cfDNA percentages stratified by result. (A) 0% to 1%, (B) 0% to 20%, and (C) biopsy occurrence (excludes one sample above the limit of quantification). Rej, rejection; w, with; w/o, without.

Table 2. Side-by-side performance characteristics between AlloSure and Prospera for all rejection at 1% threshold

Diagnostic Category	AlloSure	Prospera
Sensitivity (95% exact CI)	0.45 (0.17 to 0.77)	0.55 (0.23 to 0.83)
Specificity (95% exact CI)	0.85 (0.55 to 0.98)	0.69 (0.39 to 0.91)
PPV (95% exact CI)	0.71 (0.29 to 0.96)	0.60 (0.26 to 0.88)
NPV (95% exact CI)	0.65 (0.38 to 0.86)	0.64 (0.35 to 0.87)

PPV, positive predictive value; NPV, negative predictive value.

Table 4. Side-by-side performance characteristics between AlloSure and Prospera

Diagnostic Category	AlloSure	Prospera
AUC ROC (95% CI)	0.73 (0.52 to 0.95)	0.75 (0.54 to 0.95)

AUC ROC, area under the receiver operating characteristic curve.

Differences between the assays matter most for accurately detecting all types of rejection. Prospera missed three cases of TCMR (grade 1A; TCMR1A) using the 1% threshold that were detected by AlloSure using its published guidance of 0.5%. Even at 0.5%, Prospera missed two TCMR1A cases, which were identified by AlloSure.

The most meaningful difference captured in this study is the amount of time from sample collection to the availability of the reported results. Figure 3 displays the distribution and cumulative distribution of the difference in TAT between the paired samples. Of the paired samples, 75% had a difference of at most −1 day, indicating 75% of the AlloSure tests were reported at least 1 day earlier than Prospera tests (P<0.01, Wilcoxon signed rank test, Figure 3). The largest difference for two samples was 5 days. There were three samples where the AlloSure samples took longer to report than the paired Prospera samples, with a difference of a single day.

Discussion

Although AlloSure and Prospera both use the novel clinical parameter of measuring the percentage of dd-cfDNA to great precision, our data remains consistent with the literature surrounding AlloSure and differs slightly from the claims for the reported performance of Prospera. As suggested by Grskovic et al. (12), this may be due to the study design of the Prospera validation study. In addition, results demonstrated AlloSure has a superior TAT from blood draw to patient result.

The concept of more being better is one we are all programmed to believe, yet this report supports the focused approach of AlloSure, confirms it is not outperformed by the more complex methodology used by Prospera, and in fact is more accurate in clinical interpretation and significantly faster to gain patient results. Further to this, both tests were drawn at the same time, both have similar workflows for ordering, and both are dependent on courier-based services. In this regard, the superior AlloSure TAT makes it much more accessible in terms of making clinical decisions that guide patient care.

Assessing the overall performance of both, the AUC of the ROCs were not statistically different. This is important because diagnostic differences between assays would need very large numbers to be able to differentiate, yet the clinically relevant implications show the published data supporting AlloSure are more robust, with more clinical utility.

Prospera missed three cases of TCMR1A using the 1% threshold which were detected by AlloSure using its published guidance of 0.5%. At current diagnostic cutoffs, there was no significant difference in sensitivity, specificity, negative predictive value, or positive predictive value of AlloSure versus Prospera in detecting rejection. Although not statistically significant, the clinical interpretation of TCMR1A is critical to clinical management of patients. The data published to guide interpretation of TCMR1A is much more robust for AlloSure and is consistent with our experience (9).

This series has limitations due to an underpowered sample size, pilot experience, and single time point used in each patient, which was done to preserve patients from additional and unnecessary blood work. A larger, prospective, multicenter study is needed to validate these findings; however, these results suggest there may be little to gain. It is evident as more data are generated that cross walking published dd-cfDNA data across different platforms may be ineffective and that although dd-cfDNAs are similar, they are not the same. This is increasingly important when managing patient populations, to allow consistent comparison using a single assay.

Table 3. Side-by-side performance characteristics between AlloSure and Prospera for cellular rejection at 0.5% and 1.0% threshold

Diagnostic Category	0.5% Threshold		1.0% Threshold	
	AlloSure	Prospera	AlloSure	Prospera
Sensitivity (95% exact CI)	0.76 (0.40 to 0.97)	0.56 (0.21 to 0.86)	0.44 (0.14 to 0.79)	0.44 (0.14 to 0.79)
Specificity (95% exact CI)	0.53 (0.27 to 0.79)	0.53 (0.27 to 0.79)	0.80 (0.52 to 0.96)	0.60 (0.32 to 0.84)
PPV (95% exact CI)	0.50 (0.23 to 0.77)	0.42 (0.15 to 0.72)	0.57 (0.18 to 0.90)	0.40 (0.12 to 0.74)
NPV (95% exact CI)	0.80 (0.44 to 0.97)	0.67 (0.35 to 0.90)	0.71 (0.44 to 0.90)	0.64 (0.35 to 0.87)

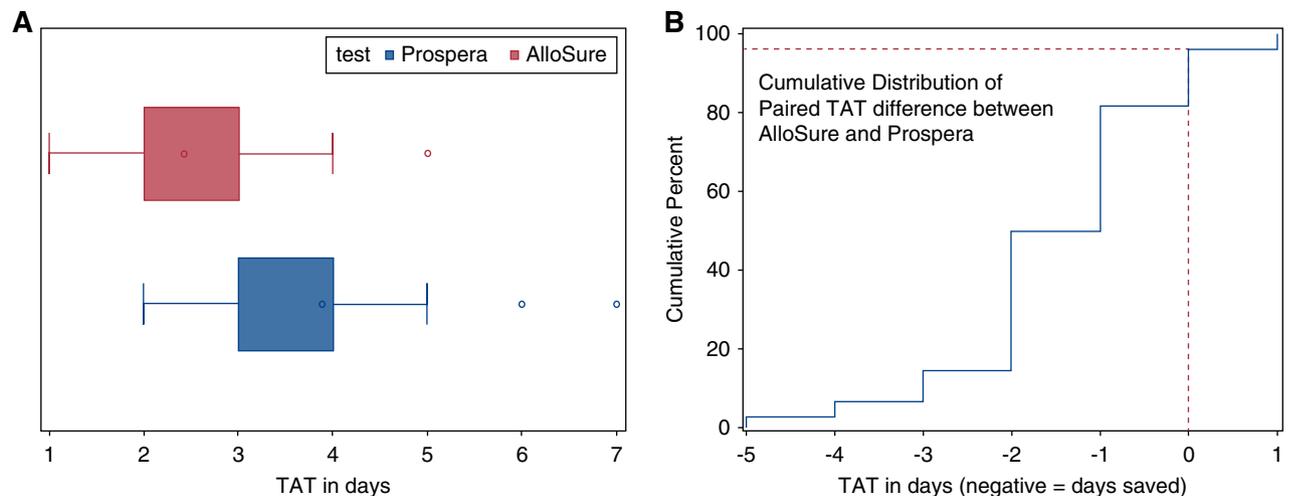PPV, positive predictive value; NPV, negative predictive value.

Figure 3. | Two-part panel showing the turnaround time and cumulative distribution of the difference (AlloSure versus Prospera) in turnaround time (TAT) between the assays.

Considering growing evidence, reevaluation of protocols to include dd-cfDNA monitoring has a place to support patients as part of their post-transplant surveillance. However, with the wide adoption of dd-cfDNAs and the potential for further assays entering the field, a clear understanding of the technology and evaluation of clinical validation data supports the importance in remaining consistent to a single platform.

More importantly, the published supporting evidence, use of real-life data, and the need for rapid turnaround to guide patient management is critical when considering clinical adoption. Both dd-cfDNA platforms are useful and provide important adjuncts in the transplant clinician's armamentarium. We believe that the use of dd-cfDNA monitoring will continue to expand clinically and that its future accuracy and precision will increase, but this needs to be supported by extensive clinical evidence and data when considering its implementation into a clinical program.

Author Contributions

A. Khalil, M. Lerman, and J.K. Melancon conceptualized the study, and were responsible for data curation and investigation; A. Khalil and M. Lerman were resonsible for validation; and J.K. Melancon was responsible for formal analysis, wrote the original draft, and reviewed and edited the manuscript.

Supplemental Material

This article contains the following supplemental material online at http://kidney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.0003512020/-/DCSupplemental.

Supplemental Table 1. Patient Cohort Demographics.

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See related editorial, "Donor-Derived Cell-Free DNA: Is It All the Same? The Jury Is Still Out" on pages 1038–1039.

Supplemental Data

Supplement Table 1. Patient Cohort Demographics

Baseline Characteristics	Result
Gender (M: F)	39:37
Race (AA: Asian: Caucasian)	50:16:10
Age (Median IQR)	49 (38.5- 64)
BMI (Median, IQR)	27 (18-37)
PRA (median, IQR)	30.0 (0.0-96.0)
Total of HLA mismatches <1: >1	21:55
Dialysis (Hemo: Peritoneal)	60:16
Cause of ESRF Hypertension Diabetes FSGS IgA Polycystic Kidney Disease	41 21 7 6 1
Total number of Patients	76