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Donor-Derived Cell-Free DNA: Is It All the Same? The Jury Is Still Out

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Monitoring kidney transplant recipients for evidence of rejection is essential to mitigate the risk of graft failure. Donor-derived cell-free DNA (dd-cfDNA) is a dynamic marker of cell turnover in the allograft that can be used as a surrogate marker of allograft injury and allows for noninvasive monitoring (1–3). Quantification of the dd-cfDNA fraction requires differentiation of donor and recipient genomes. Early methodologies relied on detection of genes found on the Y chromosome, which limited use to women recipients with men organ donors, or genome sequencing, which required separate genotyping of the donor (4). Targeted next generation sequencing (NGS) techniques use panels of single-nucleotide polymorphisms (SNPs) to differentiate dd-cfDNA from recipient cell-free DNA. Currently available NGS platforms for dd-cfDNA fraction quantification include AlloSure (CareDx, Inc., Brisbane, CA) and Prospera (Natera, Inc., San Carlos, CA). Although both rely on panels of SNPs, their methodologies are distinct. AlloSure previously used a panel of 266 SNPs selected from across all 22 somatic chromosomes with sufficient separation and low linkage (5), and it has since been updated to include 405 SNPs as noted in the recent publication by Melancon *et al*. (6). Prospera uses 13,392 SNPs concentrated across four chromosomes (3). The latter was adapted for use in kidney transplantation from an approach developed for noninvasive prenatal monitoring. Both of these tests allow for the measurement of dd-cfDNA in kidney transplant recipients without requiring knowledge of donor genotypes.

In his recent publication, Melancon *et al*. (6) reported results from simultaneous AlloSure and Prospera tests performed in 76 patients undergoing kidney allograft biopsy at two centers; 11 (14%) had rejection. An important and new finding from the analysis is that the measurements of dd-cfDNA fraction between the testing methods for the paired samples were not statistically different, although numerically, Prospera values were noted to be slightly higher than AlloSure. The assessment of the figures provided (figure 2, A–C in ref. 6) suggests that this difference was likely more noticeable at the higher dd-cfDNA fraction values (>1%), where the exact value is less likely to be relevant for clinical decision making. This information suggests that either test can be a useful screening tool; however, if the dd-cfDNA fraction is being serially monitored (for instance, in a recipient underdoing rejection therapy), switching between dd-cfDNA platforms may yield disparate results.

A second important finding is that there was no statistically significant difference between the test characteristics, including the sensitivity, specificity, positive predictive value, and negative predictive value, between AlloSure and Prospera using 1% threshold for both. However, neither test had great sensitivity for detection of rejection. In this study, the currently published threshold of 1% to define positivity of AlloSure yielded sensitivity of 45% (five of 11), lower than the 59% reported in the Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients (DART) study (1). The currently recommended threshold of 1% to define positivity of Prospera had a sensitivity of 54% (six of 11), also lower than the published 89% statistic (3).

Although a detailed review of the different types and grades of rejection identified in this study is not reported, the author notes the following (6): "Prospera missed three cases of T cell–mediated rejection 1A (TCMR1A) using the 1% threshold which 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." These comments are worthy of further discussion. The currently published threshold to define positivity of both these tests is 1% (7,8). The 0.5% cutoff for AlloSure reported in the paper by Melancon *et al*. (6) is on the basis of a recent study by Stites *et al*. (9) discussed below; however, this threshold is not a particular cutoff recommendation in the official information packet by CareDx, Inc. (7). Therefore, using different thresholds to compare test characteristics would not be expected to provide balanced information. It is quite possible that with time, both companies will change their diagnostic thresholds, reflecting different levels of allograft injury.

Additionally, in terms of the diagnosis of TCMR, the DART study documented that the performance characteristics of AlloSure were stronger for antibody-mediated rejection compared with TCMR and that AlloSure did not reliably identify patients with TCMR type 1A rejection (1). In fact, the median dd-cfDNA in the five patients with TCMR only type 1A was only 0.2% compared with 0.3% in the no rejection group.

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These findings were corroborated in a subsequent external validation study by Huang *et al.* (2) of 63 kidney transplant recipients who underwent simultaneous biopsy and AlloSure testing (34 of them had rejection) . The median dd-cfDNA in the isolated TCMR group was 0.27% compared with 0.38% in the no rejection group. Seven of the ten patients with isolated TCMR, including three with TCMR1A, had dd-cfDNA levels ≤0.35%. A more recent study by Stites *et al.* (9), used as a reference for the AlloSure threshold of 0.5% in the paper by Melancon *et al*. (6), examined 79 recipients with TCMR1A/borderline and showed that not all TCMR1A/borderline rejections are equal and that despite similar treatment, clinically adverse outcomes are associated with AlloSure dd-cfDNA ≥0.5% compared with <0.5% (9). This manuscript suggested that dd-cfDNA may be a useful tool for risk stratification in lower-grade TCMR. However, the study was not designed to assess performance characteristics or identify an appropriate threshold to define a positive result in TCMR. In comparison, a Prospera dd-cfDNA >1% could identify rejection in all five patients with TCMR1A (supplemental table 5 in ref. 3). However, because of differences in sample size, methodology, variable rates and types of rejection, and overall study design, it cannot be deciphered whether one test is better than the other.

The turnaround time of a test is an important practical variable to consider, although not a characteristic of the test itself. Although the AlloSure turnaround was faster in this study, it is unclear whether the author's center was aware that expedited shipping is available for both tests, as indicated on CareDx, Inc. and Natera, Inc. websites.

In summary, the paper by Melancon *et al*. (6) has the merit to be the first study comparing the performance of the two dd-cfDNA NGS platforms using paired samples from patients undergoing simultaneous kidney biopsies. Although no statistical difference in the performance of the two tests in the diagnosis of rejection was detected, it is important to note that the study was not powered to detect a difference. As the transplant community learns more about the clinical use of dd-cfDNA, it is important to note that temporal changes in dd-cfDNA, regardless of the platform used, may be as or more important than defining a threshold to define positivity, just as with serum creatinine, which is the most commonly used biomarker of kidney function. Another consistent finding from all of the studies investigating the performance characteristics of the dd-cfDNA tests is the modest sensitivity for detection of rejection. There are numerous other blood- and urine-based biomarkers to assess allograft function in the pipeline (10), and further studies to explore how these different tests, alone and in combination, aid with noninvasive monitoring and prognostication of the renal allograft are eagerly awaited.

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Author Contributions

N. Garg wrote the original draft and reviewed and edited the manuscript.

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