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# Letters

## Noninvasive Assay for Donor Fraction of Cell-Free DNA in Pediatric Heart Transplant Recipients

There is a compelling clinical need for a noninvasive alternative to endomyocardial biopsy (EMB) for the surveillance of rejection in heart transplant recipients. We and others have reported on donor cell-free deoxyribonucleic acid (cfDNA), which is shed from the donor allograft and is elevated during acute rejection (1,2). Barriers to clinical adoption have included the inability of previously described assays to accurately quantify cfDNA and donor fraction (DF) in a rapid, high-throughput, cost-effective manner (3). We present our experience with a clinically practical, targeted assay for the quantification of DF for identifying heart transplant recipients at low risk for cellular rejection using 2 distinct methods: Method 1, in which genotyping is performed on both recipient and donor DNA sources, and Method 2, which does not require isolated donor DNA sources for genotyping.

We obtained blood samples from 88 subjects before EMB and reported DF as a percentage of total circulating recipient cfDNA. Mean age at blood sample was 12.7 ± 8.1 years (range 0.1 to 30.2 years). Fundamental cardiac diagnosis was cardiomyopathy in 42.0% and congenital heart disease in 56.8%. A total of 59.0% of subjects were male, and 69.3% (61 of 88) were Caucasian.

Among 158 biopsy-associated samples (148 of which were asymptomatic surveillance biopsies), 134 were associated with cellular rejection grade 0 (CR0), 21 with cellular rejection grade 1 (CR1), 3 with cellular rejection grade 2 (CR2), and 0 with cellular rejection grade 3 (CR3). Using Method 1, DF increased across rejection grades: 0.11% (interquartile range [IQR]: 0.06% to 0.21%) in CR0-associated samples, 0.37% (IQR: 0.15% to 0.72%) in CR1-associated samples, and 0.97% (IQR: 0.88% to 1.06%) in CR2-associated samples. Comparing CR0 (0.11%; IQR: 0.06% to 0.21%) to CR1 or CR2 (0.48%; IQR: 0.19% to 0.89%), $p = 0.02$. Using Method 2, DF also increased across rejection grades: 0.25% (IQR: 0.17% to 0.39%) in CR0-associated samples, 0.89% (IQR: 0.44% to 5.35%) in CR1-associated samples, and 1.22% (IQR: 1.04% to 5.18%) in CR2-associated samples. Comparing CR0 (0.25%; IQR: 0.19% to 0.39%) to CR1 or CR2 (1.05%; IQR: 0.47% to 5.26%), $p < 0.001$. Receiver-operating characteristic curves with optimal cutpoints were determined and shown in **Figure 1A**.

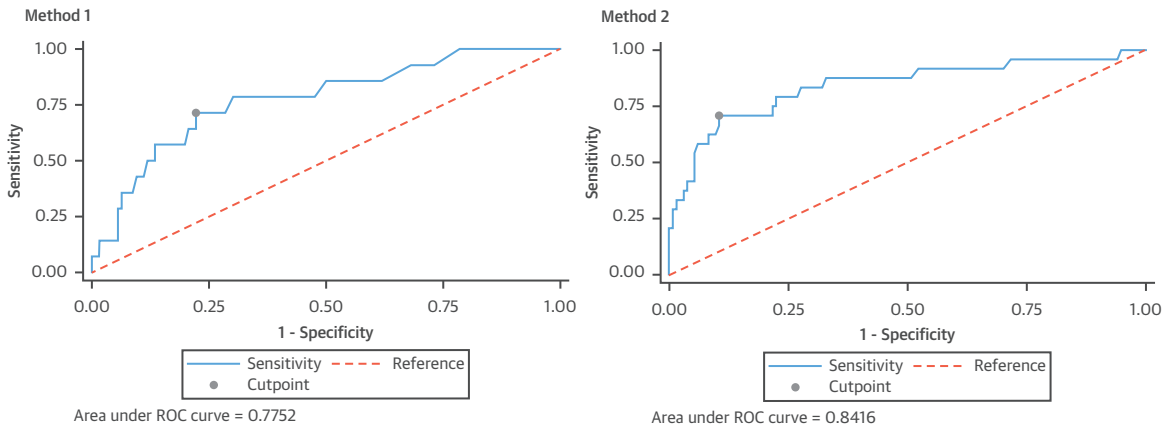
We collected 116 blood samples from 66 subjects within 24 h before selective coronary angiography. Eleven samples demonstrated cardiac allograft vasculopathy (CAV) (seven grade 1, two grade 2, and three grade 3), and 105 showed no CAV. Using Method 1, DF was 0.09% (IQR: 0.06% to 0.20%) for samples not associated with CAV and 0.47% (IQR: 0.27% to 0.71%) for CAV-associated samples ($p = 0.05$). Using Method 2, DF was 0.27% (IQR: 0.16% to 0.52%) for samples not associated with CAV and 0.55% (IQR: 0.38% to 1.22%) for CAV-associated samples ($p = 0.057$).

We next analyzed the association with acute cellular rejection and cardiac allograft vasculopathy at specified DF cutpoints (**Figure 1B**). Analyzing all 140 samples with donor genotype by Method 1 at a DF cutpoint of 0.2% and comparing CR0 to CR1 and CR2 ($p = 0.0022$). Analyzing all 158 samples by Method 2 at a DF cutpoint of 0.2% and comparing CR0 to CR1 and CR2 ($p = 0.0141$). Analyzing 102 samples associated with angiography at a DF cutpoint of 0.2%, 7 samples were true positives for CAV, 1 sample was false negative, 70 were true negatives, and 24 were false positives ($p < 0.001$). By Method 2, analyzing 116 samples at a DF cutpoint of 0.2%, 11 samples were true positive for CAV, 0 were false negative, 38 were true negative, and 67 were false positive ($p = 0.015$).

We found a targeted assay for the quantification of DF has exquisite sensitivity in ruling out the presence of acute cellular rejection in heart transplant recipients. DF increased from CR0- to CR1- to CR2-associated biopsies, suggesting its ability to detect progressive injury to the donor organ. Additionally, elevations in DF correlate with CAV. These findings suggest that precise quantification of DF is possible in clinical practice and that the observed similarity in results between both assay methods demonstrate that accuracy in quantification is

**FIGURE 1** DF cfDNA Versus CR Grade (CR0 vs CR1 or CR2) and CAV

A




B

| Method 1 | | | | Method 1 | | | | Method 2 | | | | Method 2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Outcome | < 0.2% | ≥ 0.2% | p-value* | Outcome | < 0.8% | ≥ 0.8% | p-value* | Outcome | < 0.2% | ≥ 0.2% | p-value* | Outcome | < 0.8% | ≥ 0.8% | p-value* |
| Rejection | | | | Rejection | | | | Rejection | | | | Rejection | | | |
| CR0 | 90 | 36 | 0.0022 | CR0 | 117 | 9 | 0.0057 | CR0 | 46 | 88 | 0.0141 | CR0 | 123 | 11 | <0.001 |
| CR1+CR2 | 4 | 10 | | CR1+CR2 | 9 | 5 | | CR1+CR2 | 2 | 22 | | CR1+CR2 | 10 | 14 | |
| Cardiac Allograft Vasculopathy | | | | Cardiac Allograft Vasculopathy | | | | Cardiac Allograft Vasculopathy | | | | Cardiac Allograft Vasculopathy | | | |
| No CAV | 70 | 24 | <0.001 | No CAV | 85 | 9 | 0.2065 | No CAV | 38 | 67 | 0.0153 | No CAV | 90 | 15 | 0.373 |
| CAV | 1 | 7 | | CAV | 6 | 2 | | CAV | 0 | 11 | | CAV | 8 | 3 | |

* 2-sided Fisher's exact test

Donor fraction (DF) cell-free deoxyribonucleic acid (cfDNA) in patients undergoing endomyocardial biopsy or coronary angiography. **(A)** The association of DF cfDNA with cellular rejection (CR) grade (CR0 vs. CR1 or CR2) by Method 1 (with donor genotype) and Method 2 (without donor genotype) with receiver-operating characteristic (ROC) curves. **(B)** Association of DF cfDNA with CR and cardiac allograft vasculopathy (CAV) at 2 specific cutpoints, 0.2% and 0.8% (2-sided Fisher exact test). CAV = cardiac allograft vasculopathy.

preserved when donor DNA is not available for genotyping.

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Please note: Dr. Stamm and Ms. Mahnke are employees of TAI Diagnostics. Dr. Stamm, Ms. Mahnke, and Dr. Liang own stock in TAI Diagnostics. Drs. Tomita-Mitchell and Mitchell developed the assays used in this study and are co-founders of TAI Diagnostics. Dr. Zangwill has served as a consultant for and owns stock in TAI Diagnostics. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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