4-1-2014

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Accepted version. *Journal of the American College of Cardiology*, Vol. 63, No. 12 (April 2014): 1224-1226. DOI.

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Highly Sensitive Noninvasive Cardiac Transplant Rejection Monitoring Using Targeted Quantification of Donor-Specific Cell-Free Deoxyribonucleic Acid

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To the Editor:

Approximately 20,000 cardiac transplant recipients currently reside in the United States. Rejection remains a major cause of graft failure and requires lifelong surveillance. The current gold standard for monitoring rejection is catheter-based endomyocardial biopsy (EMB), which is associated with risk and expense (1). Donor-specific cell-free deoxyribonucleic acid (DScf-DNA) has been proposed as a marker for cellular injury caused by rejection (2). Shotgun whole-genome sequencing (WGS) has been used to detect DScf-DNA (3). The complexity and cost of the analysis required by WGS limits its application as a surveillance tool. We have employed *targeted* quantitative genotyping to determine the %DScf-DNA. The targeted approach relies on selected highly-informative genomic regions and potentially provides a rapid (24 to 48 h) cost-efficient (significantly less than whole genome sequencing) method suitable for clinical surveillance. We applied this method to detect DScf-DNA in pediatric cardiac transplant recipients in a prospective blinded study.

Cardiac transplant recipients followed at the Herma Heart Center at the Children's Hospital of Wisconsin were invited to participate. Blood samples (5 ml) were collected in 3 clinical scenarios:

1. **Scheduled surveillance EMB** from *asymptomatic* heart transplant recipients in the catheterization laboratory immediately before scheduled surveillance EMB.
2. **Unscheduled diagnostic EMB** from *symptomatic* heart transplant recipients before unscheduled diagnostic EMB.

3. **Rejection** from heart transplant recipients with biopsy- proven rejection (> The International Society for Heart and Lung Transplantation grade 2R or antibody mediated rejection 1) before initiation of treatment, during treatment, and at 1 week.

Clinical, laboratory, cardiac catheterization, and echocardiographic data were recorded. Anticoagulated blood was collected to measure cell-free deoxyribonucleic acid (cf-DNA) levels. Plasma separation, cf-DNA extraction, and quantification of total cf-DNA were carried out as previously described (4). Genomic DNA for genotyping was prepared from 1 buffy coat of each recipient, and donor DNA was obtained from the Blood Center of Wisconsin. Determination of the %DScf-DNA in recipient plasma was performed using Digital Analysis of Selected Regions (DANSR, Ariosa Diagnostics, San Jose, California) (5). Genotyping of donor and recipient genomic DNA was carried out by the same assay. Loci are informative when recipient genotypes are homozygous and donor genotypes are either heterozygous or homozygous for the other allele. The minor allele frequency for informative loci was modeled as a binomial distribution. The %DScf-DNA was defined as the peak from this modeling. Summary statistics included median and range. Unpaired samples (i.e., rejection group vs. surveillance group) were compared using a Mann-Whitney *U* test. Rejection samples were compared with a Friedman analysis of variance. A Pearson correlation summarized correlations. A p value <0.05 was considered significant.

Fifty-three samples from 32 patients were analyzed.

Scenario 1

The cf-DNA levels were obtained from 26 patients undergoing 38 scheduled surveillance EMBs (Fig. 1A). Thirty-two (84%) samples contained <1% DScf-DNA. No patient with DScf-DNA <1% had pathological rejection. DScf-DNA levels exceeded 1% in 6 samples, and the highest percentage of DScf-DNA was associated with asymptomatic biopsy-proven rejection. The remaining 5 samples had negative biopsies.

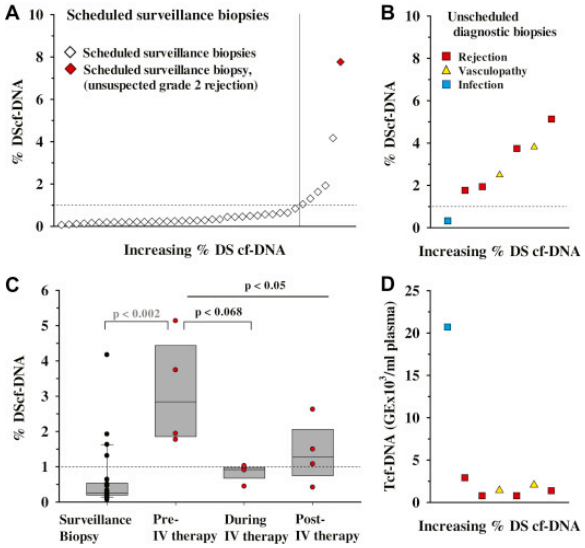

Figure 1. cf-DNA in Patients Undergoing Surveillance EMB and During Rejection

Percent donor-specific cell-free deoxyribonucleic acid (DScf-DNA) in scheduled surveillance endomyocardial biopsy (EMB) **(A)** and unscheduled diagnostic EMB samples **(B)**. **(C)** Boxplot

of surveillance EMB samples **(black circles)** and rejection samples **(red circles)** collected at 3 time points. **(D)**Total cell-free deoxyribonucleic acid (cf-DNA) in unscheduled diagnostic EMB samples. Data in **A, B, and D** are sorted on the x-axis according to increasing %DScf-DNA. Samples in **B and D** align vertically. The **dashed line in A, B, and C** highlights the 1% DScf-DNA level. The **vertical solid line in A** orients the picture so that all samples containing <1% DScf-DNA are on the **left-hand side** and all samples >1% are on the **right**. IV = intravenous.

Scenario 2

Seven samples were obtained from 6 patients before unscheduled diagnostic EMB to rule out rejection based on clinical criteria (Figs. 1B and 1D). Six samples had DScf-DNA levels >1%, and 1 sample contained DScf-DNA <1%. Four of the 6 were associated with biopsy-proven rejection; the other 2 patients had significant coronary arteryvasculopathy on angiography. The single symptomatic patient with a low percentage of DScf-DNA had high levels of total cf-DNA (Fig. 1D), implying that the dominant pathology was global rather than confined to the donor organ. This patient was diagnosed with culture-positive sepsis, the accompanying EMB was negative for rejection, and coronary angiography was normal.

Scenario 3

Four patients with biopsy-proven rejection were analyzed. All pre-treatment samples collected at diagnosis contained DScf-DNA >1% (sensitivity 100%). Following intravenous immunosuppressive therapy, all patients demonstrated significantly decreased %DScf-DNA. Interestingly, 3 to 4 days after discontinuing augmented immunosuppression, the %DScf-DNA rebounded in 3 of 4 patients (Fig. 1C). The DScf-DNA was compared with other candidate noninvasive laboratory variables (B-natriuretic peptide, troponin, and C-reactive protein) as well as echocardiographically determined left ventricular ejection fraction in predicting rejection on biopsy; DScf-DNA had the highest sensitivity and specificity (100% and 84%, respectively).

DScf-DNA may be sufficiently sensitive to detect rejection and injury to the donor organ earlier than currently available methods. Levels of DScf-DNA fall consistently by 1 week post-transplant, which may allow for noninvasive detection of rejection in the vulnerable early post-transplant period (data not shown). A sensitive noninvasive rejection monitoring method could decrease the number of biopsies needed over a lifespan, thereby considerably decreasing complications, discomfort, and cost. We were able to detect all rejection episodes, including both cellular and antibody-mediated rejection, at the earliest onset and even before clinical indicators of disease. However, these results are based on a limited sample size. A larger validation study is needed.

In summary, targeted quantitative genotyping was employed to determine circulating levels of DScf-DNA in pediatric heart transplant recipients. The percentage of DScf-DNA was elevated in all patients diagnosed with rejection. Further, all patients with DScf-DNA levels <1% were shown by biopsy and clinical parameters to be negative for rejection (negative predictive value 100%). Targeted quantitative genotyping of circulating DScf-DNA constitutes a sensitive, rapid, and cost-effective noninvasive tool potentially suitable for rejection surveillance as an alternative to EMB.

References

1 S.G. Pophal, G. Sigfusson, K.L. Booth, *et al.* **Complications of endomyocardial biopsy in children.** J Am Coll Cardiol, 34 (1999), pp. 2105-2110

2 Y.M. Lo, M.S. Tein, C.C. Pang, C.K. Yeung, K.L. Tong, N.M. Hjelm. **Presence of donor-specific DNA in plasma of kidney and liver-transplant recipients.** Lancet, 351 (1998), pp. 1329-1330

3 T.M. Snyder, K.K. Khush, H.A. Valantine, S.R. Quake. **Universal noninvasive detection of solid organ transplant rejection.** Proc Natl Acad Sci U S A, 108 (2011), pp. 6229-6234

4 M. Hidestrand, R. Stokowski, K. Song, *et al.*. **Influence of temperature during transportation on cell-free DNA analysis.** Fetal Diagn Ther, 31 (2012), pp. 122-128

5 A.B. Sparks, E.T. Wang, C.A. Struble, *et al.* **Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy.** Prenat Diagn, 32 (2012), pp. 3-9