Proficiency Testing for and Surveillance of Heart Transplant Health Using Donor-Derived Cell-Free DNA

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Introduction
Cell-free DNA (cfDNA) has been widely adopted as a diagnostic biomarker in prenatal testing and is being investigated for application in screening and recurrence testing in oncology and organ transplantation. cfDNA comprises nucleosomal protected genomic segments, most being short (~160 bp) DNA fragments released as a consequence of cell death. cfDNA is readily obtained from plasma and can be quantified despite the low abundance and even lower abundance of the subset of molecules targeted for distinct quantification.

Following solid organ transplantation, ongoing monitoring of allograft health is required for individualization and optimization of immunosuppressive therapy. Under-immunosuppression can lead to rejection of the transplanted organ and over-immunosuppression can lead to complications such as infection and cancer. For heart transplant recipients, non-invasive monitoring for acute cellular rejection (ACR) using the FDA-cleared gene expression test, ACRMap, has been widely adopted. However, there are no similar tests available for other solid organ transplants and ACRMap does not address all of the needs for heart transplant monitoring. Donor-derived cell-free DNA (dd-cfDNA) is a biomarker for allograft rejection and the resulting allograft damage with potential to detect solid organ transplanting. Reference panels for dd-cfDNA have been developed by our laboratory and others and are used to align reads to the SNP regions and determine the number of reads representing each SNP allele. This assay has an upper limit of 25% for dd-cfDNA and has been used to quantify dd-cfDNA in recipients without prior knowledge of donor or recipient genotype.

Materials and Methods

CARGO II: Multi-center European study sponsored by CareDx.

Endpts

1. dd-cfDNA Assay Design
268 SNPs with high minor allele frequency, low amplification error, low linkage, minimal polymorphism, and unbiased regarding ancestral heritage were selected for amplification and sequencing. A dd-cfDNA assay with minimal proportional or systematic bias.

2. dd-cfDNA Assay Performance

Results: 1. The dd-cfDNA Assay Identifies Low Amounts of ‘donor’ cfDNA with High Linearity and Accuracy

4. Clinical Samples Have Sufficient dd-cfDNA to Be Measured by the Assay

5. dd-cfDNA Assay Performance Characteristics Match the Distribution of dd-cfDNA in Plasma from Heart and Kidney Transplant Recipients

80% of clinical samples had dd-cfDNA concentrations greater than 3ng, 90% greater than 8ng, and 25% greater than 16ng.

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Conclusions

The precision and accuracy of the clinical-grade NGS dd-cfDNA assay for use in organ transplantation was demonstrated using custom designed reference material panels independently verified by digital PCR. Results from a large set of clinical samples are well within the performance characteristics of the assay, with critical differences between rejection and non-rejection within the linear range of detection. Successful treatment was defined by pathology grade improvement to 0R (no histopathologic damage observed).

6. Increased dd-cfDNA Correlates with Biopsy-proven Transplant Rejection

7. dd-cfDNA Decreases Following Successful Rejection Treatment

dd-cfDNA was measured in patient samples from two clinical studies. Samples from patients experiencing moderate-severe rejection as determined by biopsy pathology were compared to samples from patients with no evidence of rejection. Measured dd-cfDNA was increased in rejection-associated samples (1.9-fold (heart) and 4.4-fold (kidney) higher than non-rejection).

8. dd-cfDNA was measured in longitudinal samples from two heart transplant patients (CARGO III). The dd-cfDNA is reduced following therapeutic intervention (3 doses of 250mg prednisone at the time shown in the blue arrow). Individual visits are shown as circles (no rejection or minimal rejection from samples (moderate rejection) and squares for samples (no rejection) annotated with the biopsy pathology grade (ISHL 2004 system). Successful treatment was defined by pathology grade improvement to 0R (no histopathologic damage observed).

9. Clinical Application of dd-cfDNA

The performance of the assay matches the distribution of dd-cfDNA in plasma from heart and kidney transplant recipients.

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