DONOR-DERIVED CELL-FREE DNA (DD-CFDNA)

IMPROVING EFFICIENCY AND RELIABILITY OF POST-TRANSPLANTATION SURVEILLANCE WITH AN ULTRA-SENSITIVE NGS WORKFLOW

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Similarly to percutaneous kidney biopsies, cardiac biopsies require invasive sampling of cardiac tissue via venous catheterization in the neck, groin, or arm. Cardiac biopsy is ultrasound-assisted, or in some cases, done with the support of an echocardiogram and administration of intravenous contrast. Care of the catheter insertion site requires patient diligence for several days after the procedure. Biopsies are invasive, costly, and time- consuming for patients. Though procedures may only take hours, they often require bedrest and a reduction in physical activity for approximately one week; additionally, they require patients to travel to transplant centers, which further contributes to loss of productivity for patients and society. Furthermore, biopsies are not without risk: bleeding, pain, hematomas, infection, pneumothorax, and injury to nearby organs are among the reported complications of solid organ biopsy. In pediatric patients, avoiding biopsies is particularly attractive given the invasive nature of the procedure. Biopsies also have known reproducibility limitations. Although the development of standardized scoring has been developed at some institutions–particularly those involved in multicenter research trials–it is commonly understood that significant discordance exists among pathologists reviewing biopsied tissue. Concordance varies

depending on the degree of allograft rejection. In 2012, the Concordance Among Pathologists in the Second Cardiac Allograft Rejection Gene Expression Observational (CARGO II) Study reported only 33% concordance among pathologists reviewing higher grades of cardiac allograft rejection.

Clinical Laboratory Values as A Post-Transplantation Method of Surveillance

Clinical laboratory testing for indirect markers of transplanted tissue injury are used to reduce and/or supplement the need for invasive post-transplantation graft monitoring. These methods are safer, less expensive, and significantly decrease burden on patients; blood and urine samples are much more easily obtained than biopsied tissue. Resulting clinical laboratory values are used to assess the function of the transplanted organ but the methods measure indirect markers of tissue injury that can be slow to emerge. It is important to detect rejection as early as possible.

Grafted Organ	Metabolic and Enzymatic Markers	Additional Indicators
Kidney	Serum creatinine	Urine output, urine protein, GFR, donor-specific antibody assays
Pancreas	Urine or serum amylase, hemoglobin A1C, C-peptide level, serum lipase, glycemic load	
Liver	Alkaline phosphatase, bilirubin, aspartate and/or alanine aminotransferase	
Heart	B-type natriuretic peptide	Volatile organic compounds as oxidative stress markers

NEXT-GENERATION SEQUENCING: AN ULTRA-SENSITIVE OPTION FOR POST-TRANSPLANTATION MONITORING

Next-Generation Sequencing (NGS) is a new detection method used in the molecular diagnostics clinical laboratory. NGS is ultrasensitive and can be used to detect markers of allograft injury from an easily-obtained venous blood specimen, i.e., non-invasively. NGS has been used in the identification of biomarkers for acute allograft rejection. In 2014, Roedder, et al., introduced the kidney solid organ response test (kSORT), a correlation-based algorithm based on analysis of gene expression data in blood samples for 436 renal transplant patients in the Assessment of Acute Rejection in Renal Transplantation (AART) study. While the original kSORT relies on quantitative real-time PCR (qPCR) , it is based on a panel of 17 genetic biomarkers for acute rejection that can also be detected more sensitively by NGS.

NGS is an ideal method to detect donor-derived cell-free DNA (dd-cfDNA), a direct marker of transplanted kidney injury. Detection of dd-cfDNA requires high sensitivity, reproducibility, and efficiency for which NGS is well-suited. By using an ultra-sensitive NGS workflow, monitoring procedures are streamlined, reducing both the time and resources required to detect rejection in solid organ transplant recipients.