Donor-derived Cell-free DNA (dd-cfDNA) – The Latest Tool in the World of Pediatric Heart Transplant

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Despite continued advancements in the field of solid organ transplant, allograft rejection remains an important cause of morbidity and mortality. In adult and pediatric heart transplant recipients, endomyocardial biopsy (EMB) is currently the gold standard for diagnosing rejection. However, EMB is less than ideal due to patient discomfort, risk of complications, potential for sampling error, and inter-observer variation in result reporting. In the pediatric population, factors such as the need for anesthesia and smaller patient and organ size further increase the likelihood of complications.

Donor-derived cell-free DNA (dd-cfDNA) is a biomarker found in peripheral blood which consists of donor DNA fragments that are released into the recipient circulation when donor cells die (1). This technology allows differentiation of the donor organ DNA from the recipient DNA within the blood sample in order to provide a percentage of dd-cfDNA. At baseline, every organ has a normal rate of cell turnover, while during times of rejection, cell turnover and apoptosis are increased. Therefore, the proportion of circulating dd-cfDNA is expected to be elevated at times of rejection, making dd-cfDNA a potential direct marker for donor organ injury. It is not surprising that enthusiasm for this technology is growing as it provides a minimally invasive measure that may allow us to detect rejection and reduce the need for invasive biopsy surveillance in the near future.

Studies in adult heart transplant recipients have already demonstrated a clear association of increased percentage of dd-cfDNA with acute rejection as well as subsequent decrease following resolution of rejection with therapy (1, 2). Pediatric heart transplant data appears to demonstrate similar correlations to the adult studies, but many of these studies were not specific to pediatric patients and instead evaluated a combined cohort of both adults and children (3, 4). In adult heart transplant, research has led to the establishment of cutoff values for dd-cfDNA percentage which allow for reasonable interpretation of the test to identify rejection. Unfortunately, the volume of data is lacking in the pediatric population to establish a conclusive cutoff. Furthermore, normal cell turnover rate is potentially unpredictable in pediatric patients due to periods of rapid growth, hormonal changes, and natural immunologic changes. In spite of these challenges, dd-cfDNA has been utilized in small cohorts of pediatric heart transplant recipients to successfully detect rejection. Recently, a pediatric heart transplant center used dd-cfDNA results to decrease the frequency of EMB with no short-term adverse outcomes (5).
Not only has dd-cfDNA demonstrated potential to detect rejection in a minimally invasive manner, it has also shown potential to identify rejection earlier than our current tests. One adult study found that a rise in the percentage of dd-cfDNA can precede the development of biopsy-detectable rejection by several months (6). If it can be proven that dd-cfDNA can detect rejection earlier than current methods, the expectation would be that this would allow for early treatment of rejection which would have the long-term outcome of prolonging the life expectancy of the graft.

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In June of 2021, our institution began obtaining dd-cfDNA for our pediatric heart transplant patients at the time of cardiac catheterization and EMB. Allosure, a dd-cfDNA test provided by CareDx®, is the specific biomarker test that was utilized at our institution. Per recommendations from CareDx®, patients who were < 14 days post-heart transplant did not undergo dd-cfDNA testing. Normal percentage of dd-cfDNA on Allosure testing was defined as any level less than 0.20%. Reviewing our data from June 2021 through January 2022, a total of 19 patients had one or more samples drawn for dd-cfDNA. Of the 19 patients for whom dd-cfDNA levels were obtained, nine had abnormal values defined by the test parameters. Of those, five had evidence of acute cellular rejection (ACR) on biopsy (four with ACR 1R, one with higher grade ACR 2R). The patient with ACR 2R was

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symptomatic and had a substantial elevation in dd-cfDNA level (7.1%) at the time that rejection was identified. Subsequently, the level improved on follow up testing one month after treatment of rejection. Of the four patients with abnormal dd-cfDNA in association with negative biopsies, two had elevated filling pressures and high-risk donor specific antibodies (DSAs) and a third had elevated filling pressures and developed an acute rise in NT-proBNP that occurred six months following the testing, which then resolved following an immunosuppression increase. During this time frame, there were a total of six patients with biopsies demonstrating ACR, of which only one patient did not have an abnormal dd-cfDNA level. In this case, the biopsy findings were described as minimal with only one of four samples affected and no associated myocyte damage.

Although our data is limited at this point, it does suggest that dd-cfDNA is a relatively sensitive marker for acute cellular rejection with the only patient not captured having minimal evidence of rejection on biopsy. Although four of nine patients with an elevated dd-cfDNA level did not demonstrate rejection based on biopsy alone, there is evidence that one patient may have developed rejection six months later, and two of the patients had high-risk DSAs which may indicate ongoing biopsy-negative rejection. Thus, it is possible that almost 90% (8 of 9) of pediatric patients with abnormal dd-cfDNA had real indications of active or prospective rejection.

Advancements in immunosuppression therapy have resulted in a decrease in rates of acute rejection after solid organ transplant, but rejection continues to be a significant contributor to allograft loss. There is an urgent need to develop methods for early detection of rejection with minimal risk of complications, as this will offer the best chance for long term graft survival. Could dd-cfDNA be the answer? Our preliminary results suggest that dd-cfDNA does hold promise. We predict that over the next year more pediatric centers will start using dd-cfDNA alongside EMB, and we may soon have more insight as to whether dd-cfDNA is a consistent marker for heart transplant rejection in pediatrics. Additional validation of this test would advance the field by fostering acceptance and integration of this biomarker into clinical care with consequent reduction in need for invasive testing, thereby improving life expectancy and quality of life for this vulnerable patient population.

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