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Role of dd-cfDNA in Detection of Subclinical Rejection in Pediatric Kidney Transplant Recipients

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Abstract

Background: Detection of subclinical rejections has been a real challenge for pediatric nephrology. We hypothesized that donor-derived cell-free DNA (dd-cfDNA) in combination with serum creatinine levels and a kidney biopsy provide a "gold-standard" for clinicians so that treatment for subclinical rejection can be initiated appropriately.

Design/Methods: We performed a two-year cohort study on total (n=5) pediatric patients aged 5-19 years who received kidney transplant within 2015-2019. Blood was collected for dd-cfDNA, i.e., AlloSure, at the time of scheduled surveillance visits or when clinically indicated. During the study period, serum creatinine and tacrolimus were measured as well. For diagnosing subclinical rejection, dd-cfDNA was divided into 3 groups: low dd-cfDNA <0.5%, high dd-cfDNA 0.5%-1%, very high dd-cfDNA>1%. A kidney biopsy was performed in one patient who had very high dd-cfDNA 2.3% (nl<0.2%), high serum creatinine (sCr 1.17 mg/dL, baseline 0.5 mg/dL), in the absence of tacrolimus levels. Kidney biopsy revealed acute cellular rejection (ACR) type 1A. Patient received intravenous immune globulin (IVIG) 2 g/kg x 1, IV pulses with methylprednisolone 20 mg/kg/dose x 3, followed by a steroid taper over one month. Her serum creatinine remains normal since. Whereas in the other 4 patients, dd-cfDNA did not show significant change, no subclinical rejection was observed.

Conclusion: dd-cfDNA in combination with serum creatinine levels and a kidney biopsy can be considered the "gold standard" which improves early diagnostic utility for subclinical rejection.

Introduction

Since the 1960's, kidney transplantation has been standard practice for individuals with end stage kidney disease (ESKD), with more than 440,000 patients in the United States experiencing this procedure since 1988 [1]. It remains the ideal form of treatment for most patients with ESKD, as opposed to a life-long commitment to dialysis treatment [1-3]. Dialysis not only places a logistical burden on individuals, but also puts patients at risk of experiencing side effects from the treatment itself including mineral imbalance and decreased blood pressure. Advances have since been made in medical care for transplant patients, such as improvements in immunosuppressive agents and surgical care. However, while the rates of short-term allograft success have increased, the rates of long-term allograft success continue to remain below par of clinician's desires [4].

The Scientific Registry of Transplant Recipients found that the 10-year allograft failure rate remains 37.3% for living and 52.8% for deceased donors when analyzing kidney transplants performed in 2005 [3]. Acute and timely detection of allograft rejection and effective treatment are essential for long-term survival of renal transplant.

Following a kidney transplant procedure, transplant patients are at risk for varying severities of rejection, clinically identified as T-Cell Mediated Rejection (TCMR) (also called Acute Cellular Rejection (ACR), Antibody-Mediated Rejection (AMR), or a combination of the two [5]. Subclinical Acute Rejection (subAR) is defined as acute T- cell-mediated rejection in a patient with stable renal function, and it is associated with worse graft outcome leading to graft failure. Whereas subclinical AMR occurs less frequent, and is known to have a significant negative impact on graft outcome. Both can be detected only with invasive surveillance biopsy. Naesens M, et al. showed that up to 25% of patients with subAR was detected by protocol biopsies performed in the first year following kidney transplant, whereas overall in 35% of patients with subAR was diagnosed in the first 2 years following kidney transplant [6]. There is a need for the development of noninvasive biomarkers for kidney allograft rejection in the context of subAR.

A non-invasive screening tool, named AlloSure, has received increasing attention each year since its approval and introduction to the clinical setting in 2017. This quantitative blood test was

developed to aid in assessing allograft health in transplant patients, monitoring renal health for potential allograft rejection by measuring levels of donor- derived cell-free DNA (dd-cfDNA). Since allograft rejection involves kidney injury, as well as increase in cell death in the transplanted kidney, dd-cfDNA is released into the bloodstream to be eliminated as waste and can therefore be measured quantitatively [4]. Ideally, measuring levels of non-invasive biomarkers such as AlloSure should allow for detection of subAR, while also decreasing the number of biopsies a patient has to experience and preventing negative protocol biopsies which occur 75%-80% of the time in transplant centers that perform protocol biopsies [5].

In this study, we thought to test the hypothesis that measuring dd-cfDNA levels, in combination with serum creatinine levels and performing a kidney biopsy, provides a "gold standard" protocol for monitoring pediatric kidney transplant patients. We found that AlloSure can be used as a noninvasive marker which is sensitive enough to detect subAR so that kidney biopsy was performed, and treatment can be initiated more appropriately and timely.

Designs/Methods

Patient population

A total of five (n=5) pediatric transplant recipients were enrolled in this retrospective cohort study conducted between 2019 and 2021 at the John R. Oishei Children's Hospital. Their ages ranged from 5-19 years and each received a kidney transplant between years 2015-2019. There are a total of 30-40 pediatric kidney transplant recipients. Only patients at high-risk for rejection were included in this study. Surveillance allograft biopsies are not routinely performed. Allograft biopsy was performed for dd-cfDNA rapid peaked to 2.3% (nl<0.2%) along with serum creatinine rapid peaked to 1.17 mg/dL from baseline 0.5 mg/dL), 1.3 folds increased.

dd-cfDNA monitor/immunosuppression/serum creatinine

dd-cfDNA was analyzed as AlloSure. These high-risk patients receive dd-cfDNA testing once a month, or when clinically indicated, as compared to lower risk patients who typically receive testing once every three months. Each enrolled patient received a kidney transplant, ranging between the years 2015-2019. Blood was collected each visit and measured for dd-cfDNA levels, as well as serum creatinine and tacrolimus levels.

The amount of dd-cfDNA was assessed and grouped based on the following categories: low dd-cfDNA (<0.5%), high dd-cfDNA (0.5%-1%), and very high dd-cfDNA (>1%).

Rapid increase in dd-cfDNA values indicated acute kidney injury or an increased risk for transplant rejection, while low-to- normal dd-cfDNA values suggested less of a risk for rejection. dd-cfDNA, serum creatinine, and tacrolimus immunosuppression testing were accompanied with a kidney biopsy, but the kidney biopsy was only performed in patients who had very high dd-cfDNA and high serum creatinine, in the absence of tacrolimus immunosuppression levels suggesting non-compliant.

Treatment of rejection

Elevated levels of dd-cfDNA served as an indication of allograft rejection, which was confirmed by histological analysis *via* kidney biopsy. The 2019 Banff working classification was used to classify the type of rejection indicated. Treatment for acute cellular rejection includes intravenous immune globulin 2 g/kg x 1, IV pulses with methylprednisolone 20 mg/kg/dose x 3, followed by oral prednisone 30 mg daily (1 mg/kg/day) with taper over 1 month. Serum creatinine returned to 0.5 mg/dL. Following 1 month, the patient's serum creatinine peaked to 0.93 mg/dL, without rapid increase in dd-cfDNA level; tacrolimus immunosuppression level was on-or-above level. Without kidney biopsy, patient received IV SoluMedrol pulse 20 mg/kg/dose x 3, followed by oral prednisone 30 mg daily (1 mg/kg/day) taper over one month. Serum creatinine improved and has remained within normal range ever since.

Results

Study population

Throughout the study period, all five patients received dd-cfDNA testing per out transplant protocol. One patient among the total sample of five experienced subAR, specifically ACR type 1A, while the other four patients did not experience subAR. These individuals who formed the study cohort can be divided into two groups: Group I: ACR (n=1) and Group II: No-ACR (n=4).

dd-cfDNA and rejection

The one patient, making up Group I, experienced subAR around 18 months post- transplant. This patient's dd-cfDNA measurements were graphed and can be visually interpreted in figure 1. High levels of dd-cfDNA at 1.5% is often seen immediate post- surgery suggesting surgical-related injury which subsequently declined 0.50% or less in the first three to four months of the study. The dd-cfDNA levels remained relatively stable around 0.50% until months 13 to 14. Between months 13 to 14, a rapid rise in her dd-cfDNA levels can be acknowledged, peaking to 2.0-2.5%. Approximately at month 18, serum creatinine peaked to from baseline (0.5 mg/dL) to 1.17 mg/dL at which time, kidney biopsy was performed (Figure 2), and treatment initiated soon after. Following treatment, there was a sharp drop in dd-cfDNA levels back towards her baseline.

As indicated in figure 3, dd-cfDNA measurements were measured for the four patients that did not experience subAR (Group II). Although these patients fluctuated around baseline between 2.5-3.25%, there were no peaks in dd-cfDNA observed for Group 2 throughout the entirety of the study. In the remaining three months of the study, the dd- cfDNA steadily decreases veering from baseline.

Transplant kidney biopsy

A kidney biopsy was performed for the patient in Group I who had very high dd-cfDNA (2.3%, nl <0.2%), high serum creatinine

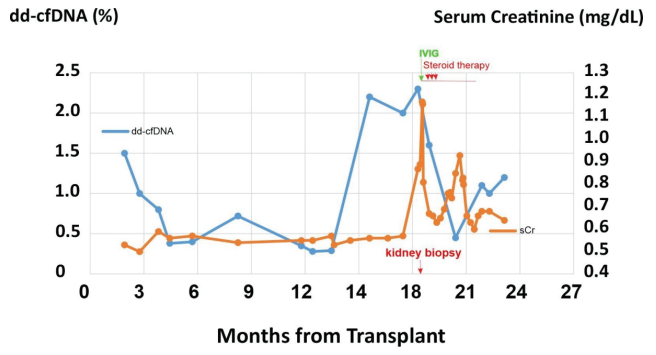

Figure 1: dd-cfDNA (%) *vs* serum creatinine (mg/dL) in the one patient with subclinical rejection over the two-year study period. Note: earlier peak of dd-cfDNA as compared to later peak of serum creatinine.

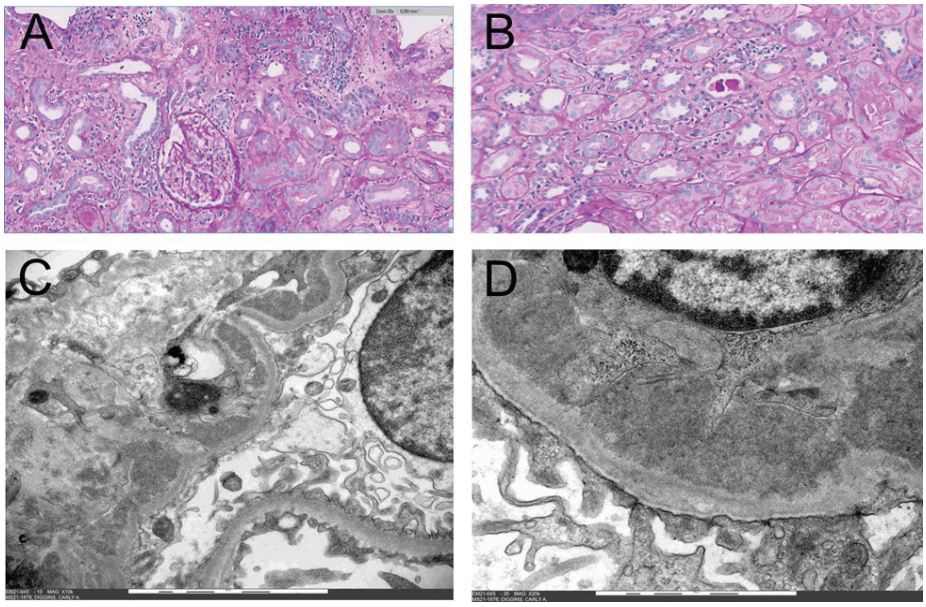

Figure 2: Kidney biopsy showing acute cellular rejection type 1A, clockwise from top left: A and B, a PAS staining that highlights inflammation in 26 to 50% of unscarred cortical parenchyma and interstitial fibrosis and tubular atrophy. C and D, an electronic microscope image that reveals there are scattered mesangial electron dense-deposits consistent with immune-complex mediated disease.

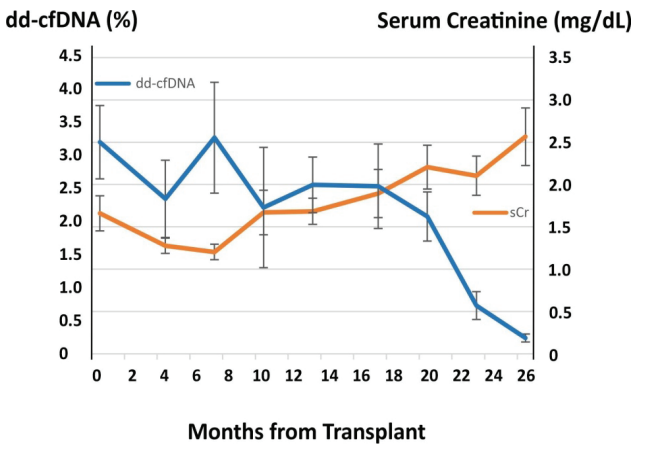

Figure 3: dd-cfDNA (%) *vs* serum creatinine (mg/dL) in the four patients (n=4) without subclinical rejection over the two-year study period.

(serum creatinine 1.17 mg/dL, baseline 0.5 mg/dL), in the absence of tacrolimus immunosuppression levels. Her biopsy sample was analyzed using the 2019 Banff Working classification. Her biopsy revealed an episode of ACR type 1A, characterized by inflammation in unscarred cortical parenchyma interstitial fibrosis, tubular atrophy, and scattered mesangial electron dense-deposits consistent with immune-complex mediated disease (Figure 2). Treatment was initiated, which is outlined above in the designs/methods section.

Serum creatinine and tacrolimus immunosuppression

Figure 1 displays that in the patient of Group I, her serum creatinine levels remained relatively stable from the beginning of the study to month 13-measuring at a stable baseline around 0.5 mg/dL. Around month 18, her serum creatinine rose rapidly to its peak at 1.2-1.3 mg/dL which was much later compared to the peak in her dd-cfDNA levels that was noted in figure 1. The 3-month-interval suggests that dd-cfDNA is a more sensitive way to measure subAR compared to serum creatinine.

Her tacrolimus immunosuppression levels, observed in figure 4, remained at target levels from post-operation period until month 9 at which time tacrolimus trough levels began to decrease, dropped to lower than target at month 13, suggesting the cause of subAR was medication noncompliance. In the absence of immunosuppressant, the patient had subAR. This is the point at which her dd-cfDNA levels would begin rising to its eventual peak between 2.0-2.5% (Figure 1). The peak of serum creatinine at 1.2-1.3 mg/dL that was observed 3 months later confirmed the subAR (Figure 1).

Discussion

It is challenging to monitor the function of transplanted organs, particularly when the transplanted organ is rejecting by the host immune system, because transplanted organs have genomes that are distinct from the recipients' genome. Cell-free DNA (cfDNA) is fragments of degraded DNA released primarily from cells undergoing apoptosis. These degraded fragments of DNA are found in circulation. Due to its rapid turnover, cfDNA circulating in blood provides a glimpse into the physiology and pathology of cells or tissues in real time [7]. CfDNA interrogation provides a powerful, yet minimally invasive, biomarker for tissue or organ injury. In transplantation, dd-cfDNA monitoring provides a tool for identifying active allograft injury at the time of transplantation, infection, and rejection [7]. In heart transplant recipients, very high levels of dd-cfDNA, particularly changes from past measurements, indicated the onset of rejection [8]. Newer technologies focus on a handful of SNPs (~266 in Allosure, ~13,000 in Prospera). Furthermore, donor genotyping is no longer required [9].

In this study, we investigated the role of dd-cfDNA in the detection of subAR in pediatric kidney transplant recipients. We selected a total

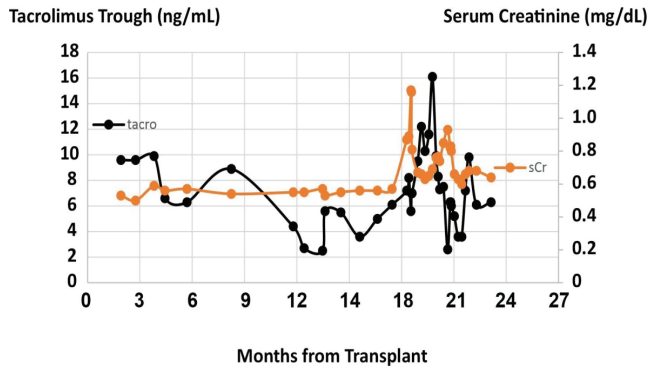

Figure 4: Tacrolimus (ng/mL) *vs* serum creatinine (mg/dL) levels in one patient with subclinical rejection over the two-year study period.

of five (n=5) pediatric patients aged 5-19 years who received a kidney transplant between years 2015-2019. We monitored patients with stable kidney function to detect "silent" or "subclinical rejection".

In the patient described in Group I, immediately following a transplant surgery, there was a 1st dd-cfDNA peak, suggesting allograft injury at the time of transplantation, which was subsequently decreased to baseline within 3-4 months of post-operation. The 2nd rapid rise in this patient's dd-cfDNA level occurred between months 13-14 which was sustained. Serum creatinine level also peaked to 1.2-1.3 mg/dL from baseline (0.5-0.6 mg/dL), however, it was not until month 18 (Figure 1). This observation reveals the ability of dd-cfDNA to detect subAR much sooner with greater sensitivity than traditional markers, such as serum creatinine, would often indicate. Her immediate kidney biopsy confirmed acute cellular rejection (Figure 2). The patient was then given IVIG 2 g/kg x 1, IV SoluMedrol pulse 30 mg/kg/dose x 3, followed by a steroid taper over one month. Her serum creatinine and dd-cfDNA levels have returned to normal since, with no complications or peaks in measurements to date. The reason for acute cellular rejection was noncompliance, as the tacrolimus trough levels at months 8-9 trended low and became undetectable at month 13 at which time dd-cfDNA peaked (Figure 4).

The four patients in Group II, those that did not experience subAR, were observed to have higher baseline serum creatinine levels overall since they received a kidney transplant as early as 2015. Furthermore, most have medication non-compliance which can also explain the higher baseline serum creatinine levels. A key point shown in figure 3 is that there are no peaks in dd-cfDNA levels. Recall that these patients did not experience subAR, nor was it clinically indicated which is why they did not receive a kidney biopsy. This lends supports the ideal ability of AlloSure to accurately assess allograft health, which could potentially reduce the number of kidney biopsies that a patient must endure [7].

In this study, we showed that dd-cfDNA is a screening test. It is not a diagnostic test. It is a better selection for confirming testing. The ability of dd-cfDNA to aid in detection of subAR gives clinicians a "head start" prior to the predicted allograft rejection, in thinking about how best to treat and also prevent subAR from occurring. Compared to serum creatinine, dd-cfDNA seems to be more sensitive. Compared to kidney biopsy, dd- cfDNA is noninvasive. However, dd-cfDNA alone is unable to determine the type of rejection occurring. Therefore, we use dd-cfDNA to eliminate biopsies when dd-cfDNA levels are below the threshold and avoid surveillant kidney biopsies which may not be necessary. When biopsies are difficult to obtain, series testing of concerning patients would be appropriate. The gold standard protocol for monitoring graft function in pediatric kidney transplantation is the combination of dd-cfDNA, serum creatinine, and percutaneous kidney biopsy.

Conclusion

In conclusion, we can use dd-cfDNA to eliminate biopsies when dd-cfDNA <1%. We also can use dd-cfDNA to assist decision making when biopsy equivocal or nondiagnostic and surveillance post-rejection therapy. We do not intend to use dd-cfDNA testing in replacing Donor Specific Antibodies (DSA), serum creatinine, or kidney biopsy. Rather, this noninvasive assay reduces the need for invasive biopsies and enables clinicians to better target biopsies to patients in whom they are truly indicated, while providing sampling uniformity, safety, cost saving, and improved patient satisfaction. Further studies are required to validate the thresholds that trigger additional testing, to determine the optimal monitoring frequency, to establish whether dd-cfDNA testing can distinguish between acute rejection subtypes, and to evaluate long-term outcomes across the variety of clinical applications mentioned.

Acknowledgement

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Authors' Contributions

Rhys Mendel performed data analysis and wrote the original manuscript. Dr. Corinne Benchimol involved in patients' care, and edited on manuscript. Dr. Xiaoyan Wu involved in patients' care, edited on manuscript, and she is the corresponding author.

Conflict of Interest

None.

Disclosures

None.

Data Availability Statement

The data that support the findings of this study are available on request form the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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