Donor Derived Cell Free DNA Kinetics Post Kidney Transplant Biopsy

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Abstract

Background:

Donor-derived cell-free DNA (dd-cfDNA) has generated interest as a biomarker for kidney transplant (KT) rejection. It is possible that the KT biopsy procedure can cause the release of dd-cfDNA, therefore affecting the reliability of this assay in the post biopsy period. In this study we evaluated the effect of KT biopsy on the kinetics of dd-cfDNA.

Methods:

We conducted a single arm prospective study. Samples were collected from 16 adult KT recipients undergoing KT biopsy. All participants had samples drawn within eight hours prior to the biopsy (pre-biopsy), within 20 minutes (hour 0), 2 hours (hour 2), and 24-48 hours (hours 24-48) after the biopsy. We evaluated the change in dd-cfDNA from the pre-biopsy time point to the following 3 time points after the biopsy.

Results:

At hour 0 and hour 2, there was a significantly larger log dd-cfDNA mean score compared to the pre-biopsy score [Least square mean (LSM) estimate 0.4 (0.17, 0.63) and 0.39 (0.09, 0.68) respectively]. By 24-28 hours post biopsy there was no significant difference in log dd-cfDNA mean score compared to the pre-biopsy score [LSM estimate -0.21 (-0.6, 0.19)].

Conclusion:

KT biopsy leads to an increase in dd-cfDNA after the procedure, however, this rise is transient and resolves by 24-48 hour after the biopsy. Providers can obtain dd-cfDNA level as soon as 48 hours post biopsy with high confidence that the levels have not been affected by the biopsy. In addition, our findings suggest possible non-traditional reasons for increase in dd-cfDNA such as mechanical reasons.

Background

Kidney transplant (KT) rejection is one of the most common causes of renal allograft loss¹. Early detection and treatment of rejection are essential to prolong renal allograft survival. Renal allograft histology obtained *via* needle biopsy remains the gold standard for diagnosis of rejection. Due to the potential risks associated with biopsy and possible inter-observer variation in assessing histopathology, search for non-invasive and more accurate methods to detect KT rejection continued in the last few decades². Plasma donor-derived cell-free DNA (dd-cfDNA) detected in the blood of KT recipients has been proposed as a noninvasive marker for diagnosis of renal allograft rejection³⁻¹¹. The Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients

(DART) study validated that plasma levels of dd-cfDNA >1% could discriminate active rejection from no rejection with a high negative predictive value of 84% and a positive predictive value of 61%³.

Up until now, the standard of care is that patients with an elevated plasma dd-cfDNA level commonly undergo a renal allograft biopsy to confirm the presence and type of rejection. However, there are no data on whether the biopsy itself affects the level of dd-cfDNA. It is possible that the biopsy itself can cause the release of more dd-cfDNA in the blood stream, therefore affecting the reliability of dd-cfDNA measurements for the diagnosis of acute rejection and response to treatment. Determining the effect of the biopsy procedure on dd-cfDNA levels is also important because the sample for dd-cfDNA may have been obtained several days prior to the biopsy. If the biopsy does cause a release of dd-cfDNA, it would be important to know the extent and duration of this change, and whether a new baseline of dd-cfDNA level develops. This information is essential for the clinician managing KT recipients with abnormal dd-cfDNA and/or rejection to assess response to therapy.

In this study, we proposed to answer these questions through an evaluation of the kinetics of plasma dd-cfDNA after renal allograft biopsy. We hypothesized that renal allograft biopsy causes an increase in dd-cfDNA level in KT recipients that will be transient but interpretable. Our secondary hypothesis is that in KT recipients undergoing renal allograft biopsy, the level of plasma dd-cfDNA returns to its baseline in a short period after the biopsy.

Methods

Study Cohort:

We conducted a single arm prospective study. The enrollment period was over ten months from May 2018 through March 2019. Patients were eligible for the study if they were adult (≥ 18 years of age) male or female KT recipients undergoing renal allograft biopsy. Written informed consent for study participation was required. Exclusion criteria were as follows: (1) multiple solid organ transplants, (2) pregnancy, (3) history of bone marrow transplant, (4) kidney transplant from an identical twin, and (5) kidney transplant less than two weeks from the time of transplantation.

The institutional review board at our institution approved the study, and all of the patients provided written informed consent. The study was an investigator-initiated trial and funded by CareDx, Inc. (Brisbane, CA)

Donor Derived Cell Free DNA Sampling and Measurement:

We used the AlloSure® assay (CareDx, Brisbane, CA) for measuring dd-cfDNA. In 2017, Centers for Medicare and Medicaid Services (CMS) approved the use of AlloSure®, a test that measures dd-cfDNA, to be used to assess the probability of renal allograft rejection¹². The AlloSure® test is a clinical-grade, targeted, next generation sequencing (NGS) assay that measures single-nucleotide polymorphisms (SNPs) to quantify dd-cfDNA in kidney transplant recipients. Blood samples for dd-cfDNA measurements

were collected from kidney transplant recipients undergoing kidney transplant biopsy. Two samples of blood were collected at the same venipuncture in Streck Cell-Free DNA BCT® tubes, stored at room temperature, and shipped to the CLIA-certified laboratory at CareDx, Inc. Participants had an intravenous catheter placed for sample collections. All participants had samples drawn within eight hours prior to the biopsy (pre-biopsy), within 20 minutes (hour 0), 2 hours after (hour 2), and 24-48 hours (hours 24-48) after the biopsy. Approximately, 20cc of blood was collected per draw for a total of 80 cc per patient.

Study Endpoints:

The study endpoint was the change in dd-cfDNA. The change was assessed over the study time points which are immediately (within 20 min), hour 2, and hours 24-48 after renal allograft biopsy.

Statistical Analyses:

We reported descriptive statistics as means $\pm$ standard deviation for normally distributed continuous variables and as median (25th and 75th percentiles [Q25-Q75]) for continuous variables with a skewed distribution. Categorical variables are expressed as frequencies (percentage). Measurements of dd-cfDNA are generally positively skewed. A natural log transform of the dd-cfDNA measurements was performed to ensure the data were more normally distributed and maximally symmetric. Repeated measures ANOVA method were used to compare dd-cfDNA among different time points. All analyses were conducted in SAS, version 9.4 (SAS Institute Inc).

Results

Study Population

Sixteen KT recipients were included. Clinical characteristics are shown in Table 1. Mean age at the time of biopsy was 50.6 ± 7.02 years. A majority of patients were men and Caucasian. Mean serum creatinine at the time of biopsy was 2.24 ± 0.42 mg/dL. The source of renal allograft was deceased donor in all recipients. The maintenance immunosuppression regimen consisted of tacrolimus, mycophenolate, and prednisone in all the recipients. All biopsies were performed for a clinical indication. The most common reason for obtaining biopsy was acute kidney injury in 12 patients, while 4 patients underwent the biopsy due to elevation in dd-cfDNA.

Table 1
Clinical Characteristics

Clinical Characteristic (n16)	
Mean age (Year)	50.62 +/- 7.02
Sex	12
Male	4
Female	
Race	9
White	7
Non-white	
Allograft source	16
Deceased donor	0
Living donor	
Number of HLA antigen mismatches	1
6	2
5	10
4	2
3	1
0	
Mean serum creatinine (mg/dL)	2.24 +/- 0.42
Maintenance immunosuppression	16
tacrolimus, mycophenolate, prednisone	
Reason for biopsy	12
Acute kidney injury	4
Rise in dd-cfDNA	
Number of tissue cores obtained	14
2 cores	2
3 cores	

Clinical Characteristic (n16)	
Location of procedure	14
Outpatient	2
Inpatient	
Post-biopsy complications	0
Hematoma	0
Arteriovenous malformation	

Biopsy Technique And Procedural Complications

All participants underwent an ultrasound guided percutaneous renal allograft biopsy using an 18-gauge automatic spring-loaded biopsy gun. We collected 2 tissue cores in 14 participants and two from whom we collected 3 tissue cores. None of the patients developed a hematoma or an arteriovenous malformation (AVM) as a result of the procedure.

Change In Dd-cfdna

Blood sampling was complete across the time points in 11 participants. Five patients had incomplete blood collections. Four had one missing value and 1 had two missing values. There were no missing values from the pre-biopsy samples but there were 2 missing values from each of the remaining time points' samples. The reasons for missing values were inability to obtain the sample and/or an inadequate sample as deemed by the processing laboratory.

The longitudinal analysis of the log transformed dd-cfDNA measurements demonstrated there were differences in measurements as a function of time (Fig. 1). The pre-biopsy time point was compared against all remaining time points. The natural log-transformed dd-cfDNA at hour 0 and hour 2 were significantly higher than that at pre-biopsy [Least square mean (LSM) estimate 0.4 (0.17, 0.63) for hour 0 and 0.39 (0.09, 0.68) for hour 2], while the natural log-transformed dd-cfDNA at hour 24–48 was not significantly higher than pre-biopsy [LSM estimate – 0.21 (-0.6, 0.19)] (Table 2). The natural log-transformed dd-cfDNA at hour 24–48 was significantly lower than at hour 0 and hour 2 [LSM estimate – 0.6 (-0.94, -0.27) for hour 0 and – 0.59 (- 0.97, -0.22) for hour 2].

Table 2
Differences of dd-cfDNA Least Squares Means between Two Time Points

Time Point	Reference Time Point	Estimate (Confidence Interval)	P value
Hour 0	Pre-biopsy	0.4 (0.17, 0.63)	0.0022
Hour 2	Pre-biopsy	0.39 (0.09, 0.68)	0.0138
Hours 24–48	Pre-biopsy	-0.21 (-0.6, 0.19)	0.2846
Hour 2	Hour 0	-0.01 (-0.18, 0.16,)	0.8854
Hour 24–48	Hour 0	-0.6 (-0.94, -0.27)	0.0017
Hour 24–48	Hour 2	-0.59 (- 0.97, -0.22)	0.0042

Discussion

Our study is the first of its kind assessing the kinetics of dd-cfDNA after renal allograft biopsy and fills the gap in knowledge about the effect of biopsy on dd-cfDNA levels. Our study demonstrates that dd-cfDNA rises after renal allograft biopsy, however, the rise is transient and returns to baseline by 24 to 48 hours confirming that the level of dd-cfDNA does not permanently change following renal allograft biopsy. Our findings can assist the transplant provider managing KT recipients in decision making regarding time of dd-cfDNA measurement in certain scenarios, and allow re-measurement as soon as 48 hours after the biopsy.

Although renal allograft biopsies are generally considered to be safe, it is associated with risks such as bleeding, hematoma, and AVM formations. Liquid biopsy obtained by measurement of dd-cfDNA can serve as an alternative to the invasive renal allograft biopsy and has evolved to a frequently used tool for surveillance and diagnosing of KT rejection, as well as monitoring of therapy ^{3, 8, 10, 13}.

The transient rise in dd-cfDNA in our study can be explained by direct renal transplant tissue injury from the biopsy needle. This injury leads to the release of donor dd-cfDNA into the blood stream. Importantly, the mean half-life of dd-cfDNA is relatively short at 30 minutes, but varies from several minutes to 1–2 hours ^{14, 15}. Clearance of dd-cfDNA depends on the rate of production and elimination. The elimination of dd-cfDNA can occur in multiple sites including the “home” tissue, blood, and other organs (liver, spleen, kidney, and lymph nodes) ^{16, 17}, hence, there are several factors affecting the efficacy of dd-cfDNA clearance. In the example of biopsy-related tissue damage, the injury, and therefore the rate of production, is transient unless a complication leading to a longer lasting injury develops. Therefore, the rise is expected to be of short duration as long as mechanisms of elimination are intact. It is worth mentioning here that the AlloSure® assay has high reproducibility within and across runs. The coefficient variation within runs is 4.6–9.2 and across run is 4.5–9.9% ¹⁸.

Our study has some limitations. We used the Allosure® assay to measure dd-cfDNA, and the results may not apply to other NGS assays that measure dd-cfDNA in kidney transplant recipients. We are pleased that none of our subjects developed a significant complication during the biopsy, however, as a result our findings cannot be generalizable to patients who develop complications such as hematoma, major bleeding, or AVM formation. The levels of dd-cfDNA might vary biologically overtime for factors not related to tissue injury ⁶. It is possible that perturbations unrelated to direct injuries to the renal allograft, such as the turnover/death rate of cells originating from the recipient's tissues, could confound the results and interpretation of dd-cfDNA. Finally, this is a single center study with a relatively small number of patients but each patient was his own control.

In conclusion, our study showed that renal allograft biopsy leads to an increase in dd-cfDNA immediately and 2 hours after the procedure, however, this rise is transient and resolves in 24–48 hours. As long as there are no complications related to the biopsy, providers taking care of KT recipients can obtain dd-cfDNA level as soon as after 48 hour after biopsy with high confidence that the levels are not affected by the biopsy. Our study also gives insight into new causes of dd-cfDNA release. It is not only released with rejection or other physiological injuries such as infection, but we have shown the novel finding that dd-cfDNA can be released after mechanical injury from a kidney biopsy and may occur after other mechanical injuries, and help to explain “false-positive” dd-cfDNA levels.

Abbreviations

AVM
arteriovenous malformation
dd-cfDNA
Donor derived cell free DNA
KT
Kidney transplant
LSM
least square mean
NGS
next generation sequencing
Pre-biopsy
Prior to the biopsy

Declarations

-Ethics approval and consent to participate:

The study was performed in accordance with the [Declaration of Helsinki](#). The study was approved by the Johns Hopkins Hospital Institutional Review Board. Written patient's consent was required to participate in the study.

-Consent for publication:

Not applicable.

-Availability of data and materials:

All data generated or analyzed during this study are available from the corresponding author on reasonable request and will be saved and available for 5 years after publication.

-Competing interests:

Daniel Brennan has received consulting fees from Amplyx, Sanofi and CareDx, speaker fees from CareDx. Johns Hopkins School of Medicine has received research support (DCB) and fellowship support (AB, MA) from CareDx.

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Other authors declare no conflicts of interest.

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-Authors contributions:

All authors have read and approved the manuscript.

YK participated in writing of the paper, performance of research, and samples collection

Anshul Bhalla participated in sample collection and performance of research

AS participated in sample collection

YJ performed data analysis

S Alakhdhair participated in sample collection

SO participated in sample collection

MA participated in sample collection and data analysis

DB participated in research design and writing of the paper

S Alasfar participated in research design, performance of research, writing of the paper, and data analysis

-Acknowledgement:

Not applicable

References

1. Djamali A, Kaufman DB, Ellis TM, et al. Diagnosis and management of antibody-mediated rejection: current status and novel approaches. *Am J Transplant*. 2014;14(2):255–71.
2. Morgan TA, Chandran S, Burger IM, et al. Complications of Ultrasound-Guided Renal Transplant Biopsies. *Am J Transplant*. 2016;16(4):1298–305.
3. Bloom RD, Bromberg JS, Poggio ED, et al. Cell-Free DNA and Active Rejection in Kidney Allografts. *Journal of the American Society of Nephrology: JASN*. 2017;28(7):2221–32.
4. Jordan SC, Bunnapradist S, Bromberg JS, et al. Donor-derived Cell-free DNA Identifies Antibody-mediated Rejection in Donor Specific Antibody Positive Kidney Transplant Recipients. *Transplant Direct*. 2018;4(9):e379.
5. Zhang H, Zheng C, Li X, et al. Diagnostic Performance of Donor-Derived Plasma Cell-Free DNA Fraction for Antibody-Mediated Rejection in Post Renal Transplant Recipients: A Prospective Observational Study. *Front Immunol*. 2020;11:342.
6. Bromberg J, Brennan D, Poggio E, et al. Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications. *The Journal of Applied Laboratory Medicine: An AACC Publication*. 2017;2:309–21.
7. Garcia Moreira V, Prieto Garcia B, Baltar Martin JM, et al. Cell-free DNA as a noninvasive acute rejection marker in renal transplantation. *Clin Chem*. 2009;55(11):1958–66.
8. Beck J, Oellerich M, Schulz U, et al. Donor-Derived Cell-Free DNA Is a Novel Universal Biomarker for Allograft Rejection in Solid Organ Transplantation. *Transplant Proc*. 2015;47(8):2400–3.
9. Beck J, Bierau S, Balzer S, et al. Digital Droplet PCR for Rapid Quantification of Donor DNA in the Circulation of Transplant Recipients as a Potential Universal Biomarker of Graft Injury. *Clin Chem*. 2013;59(12):1732–41.
10. Stites E, Kumar D, Olaitan O, et al: **High levels of dd-cfDNA identify patients with TCMR 1A and borderline allograft rejection at elevated risk of graft injury.** *Am J Transplant* 2020.
11. Huang E, Sethi S, Peng A, et al. Early clinical experience using donor-derived cell-free DNA to detect rejection in kidney transplant recipients. *Am J Transplant*. 2019;19(6):1663–70.
12. **Local Coverage Determination (LCD): MolDX: AlloSure® Donor-Derived Cell-Free DNA Test (L37303).**
13. Oellerich M, Shipkova M, Asendorf T, et al. Absolute quantification of donor-derived cell-free DNA as a marker of rejection and graft injury in kidney transplantation: Results from a prospective observational study. *Am J Transplant*. 2019;19(11):3087–99.
14. Celec P, Vlkova B, Laukova L, et al. Cell-free DNA: the role in pathophysiology and as a biomarker in kidney diseases. *Expert Rev Mol Med*. 2018;20:e1.

15. Yu SCY, Lee SWY, Jiang P, et al. High-Resolution Profiling of Fetal DNA Clearance from Maternal Plasma by Massively Parallel Sequencing. *Clin Chem.* 2013;59(8):1228–37.
16. Leung F, Kulasingam V, Diamandis EP, et al. Circulating Tumor DNA as a Cancer Biomarker: Fact or Fiction? *Clin Chem.* 2016;62(8):1054–60.
17. Kustanovich A, Schwartz R, Peretz T, et al. Life and death of circulating cell-free DNA. *Cancer Biol Ther.* 2019;20(8):1057–67.
18. Grskovic M, Hiller DJ, Eubank LA, et al. Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. *J Mol Diagn.* 2016;18(6):890–902.

Figures

Figure 1

A: Graph of the trend in percentage of donor derived cell-free DNA from pre kidney transplant biopsy time point to different time points after the biopsy. B: Box plot of the percentage of donor derived cell-free DNA at different time points (Pre- biopsy, hour 0, hour 2, and hour 24-48 after the biopsy).