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Donor-Derived Cell-Free DNA in Biopsy-Proven Antibody-Mediated Rejection Versus Recurrent IgA Nephropathy After Kidney Transplantation

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Received 22 June 2023; accepted 17 July 2023; published online 21 July 2023

Kidney Int Rep (2023) **8,** 2141–2145; https://doi.org/10.1016/j.ekir.2023.07.011

KEYWORDS: antibody-mediated rejection; biomarker; donor-derived cell-free DNA; kidney transplantation; molecular diagnostic techniques; recurrent IgA nephropathy

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INTRODUCTION

Recurrent primary disease causes graft loss in 8% of kidney transplant recipients.[1] IgA nephropathy (IgAN) is one of the most prevalent recurrent primary diseases and is associated with impaired graft function and premature graft loss.[2] Young recipient age,[S1] rapid progression of native kidney disease, close human leukocyte antigen-matching,[S2] and early steroid withdrawal[3,S3] have been described as possible risk factors for recurrent IgAN.

Although several biomarkers have been studied for risk assessment in kidney transplant recipients with primary IgAN, the lack of assay standardization failed to translate their significance into clinical benefit. Total levels of serum IgA, Gd-IgA1, and Gd-IgA1-IgG, as well as IgA-autoantibodies assessed before or at transplantation are of prognostic value and can identify kidney transplant recipients at risk[4] for recurrent IgAN, but the evidence about their utility in the posttransplant course remains scarce.[5]

Donor-derived cell-free DNA (dd-cfDNA) is increasingly recognized as a noninvasive biomarker for graft injury in kidney transplantation, especially in the context of allograft rejection.[6,7] Although several studies confirmed its ability to detect antibody-mediated rejection (ABMR),[8,S4] it has not been studied in patients with recurrent primary disease, such as IgAN, so far.

Both ABMR and recurrent IgAN are major causes of graft loss and can present with comparable clinical features such as decline in renal function, proteinuria, or worsening hypertension. Becuase ABMR and recurrent IgAN are indistinguishable using estimated glomerular filtration rate and urine albumin-to-creatinine-ratio (uACR) alone, we evaluated the ability of dd-cfDNA to discriminate both entities in consecutive cases from an ongoing prospective observational trial.

RESULTS

Patient Characteristics

At the time of clinically indicated biopsies, we collected venous blood samples in Streck Cell-Free DNA BCT tubes (Streck, NE) and measured absolute (copies/ml) and relative (%) dd-cfDNA using digital-droplet polymerase chain reaction, as previously described.[7] In total, we included 73 kidney transplant recipients with 75 biopsy-matched dd-cfDNA measurements from April 2020 until March 2023. For this analysis, we excluded biopsies showing TCMR IA ($n = 3$), severe glomerulosclerosis or fibrosis ($n = 2$), cortex necrosis ($n = 1$), infection-related graft pathology ($n = 8$), or donor specific anti-human leukocyte antigen antibody (DSA)-negative microvascular inflammation ($n = 4$) as shown in Supplementary Figure S1 and Supplementary Table S1. The dd-cfDNA results and renal function parameters for

Table 1. Baseline characteristics and clinical parameters of 57 patients at the time of clinically indicated kidney allograft biopsy

Variable	ABMR	Recurrent IgAN	No rejection
Patient count	21	15	21
Demographics			
Recipient age, yrs, median (IQR)	49 (39–63)	44 (37–53)	53 (44–68)
Sex (male vs. female)	57% vs. 43%	80% vs. 20%	67% vs. 33%
Reported cause of ESRD			
- IgAN	1 (5%)	13 (87%)	3 (14%)
- Genetic	9 (43%)	-	9 (43%)
- Other	6 (29%)	-	8 (38%)
- Unknown	5 (24%)	2 (13%)	1 (5%)
Transplantation			
Time since transplantation, mos, median (IQR)	130 (68–191)	144 (109–191)	81 (7–128)
Donor type			
- Deceased	8 (38%)	6 (40%)	10 (48%)
- Living/ABO-i	13 (62%)/1 (5%)	9 (60%)/1 (7%)	11 (52%)/4 (19%)
Induction therapy			
- Basiliximab	16 (76%)	14 (93%)	21 (100%)
- ATG	3 (14%)	1 (7%)	-
- Other	2 (10%)	-	-
Baseline immunosuppression			
- CNI-based triple IS	10 (48%)	6 (40%)	15 (71%)
- Steroid withdrawal	8 (38%)	6 (40%)	6 (29%)
- Other	3 (10%)	3 (20%)	-
Preformed anti-HLA-DSA, *n* (%)	2 (10%)	-	1 (5%)
De novo anti-HLA DSA, *n* (%)	19 (90%)	6 (40%)	8 (38%)
Other medication			
ACE inhibitor/ARB	17 (81%)	10 (66%)	14 (67%)
SGLT-2 inhibitor	2 (10%)	1 (7%)	1 (5%)
Kidney function at time of biopsy			
eGFR CKD-EPI, ml/min/1.73m2, median (IQR)	41.1 (29–53)	32 (21–38)	30 (25–39)
uACR, mg/g, median (IQR)	259 (121–921)	577 (277–1472)	34 (11–69)
Hematuria	7 (33%)	7 (47%)	3 (14%)
Dd-cfDNA at time of biopsy			
Relative dd-cfDNA, %, median (IQR)	1.68 (1.1–2.7)	0.32 (0.24–0.41)	0.30 (0.26–0.54)
Absolute dd-cfDNA, copies/ml, median (IQR)	76 (57–103)	11 (7–13)	12 (7–16)
Total cfDNA, copies/ml, median (IQR)	4027 (2691–5782)	3827 (2512–5446)	3246 (2220–4649)

ABMR, antibody-mediated rejection; ACE, angiotensin converting enzyme; anti-HLA, anti-human leukocyte antigen; ARB, angiotensin receptor blocker; ATG, anti-thymocyte globulin; CNI, calcineurin inhibitor; DSA, donor specific antibody; eGFR, estimated glomerular filtration rate; IgAN, IgA nephropathy; IQR, interquartile rangeIS, immunosuppression; SGLT-2, sodium glucose linked transporter 2; uACR (urine albumin-to-creatinine ratio).

excluded entities are summarized in Supplementary Table S2 and commented on in the Supplementary Material.

The remaining 57 dd-cfDNA-matched biopsies were assigned to 3 groups based on histopathology as follows: (i) active or chronic active ABMR ($n = 21$); (ii) recurrent IgAN ($n = 15$); and (iii) no signs of rejection, infection, or glomerulonephritis ($n = 21$). For each group, clinical characteristics, dd-cfDNA levels, and renal parameters at the time of biopsy are provided in Table 1.

The ABMR group comprises 10 cases of active and 11 cases of chronic active ABMR. Three patients (14%) had subclinical rejection as defined by stable creatinine and uACR <30 mg/g. Two patients had preexisting DSA and 19 developed *de novo* DSA, which persisted at the time of the biopsy.

From 15 patients with recurrent IgAN, 5 (33%) showed endocapillary hypercellularity, and 4 (27%) showed crescent formation as signs of severe histological recurrence, whereas 3 patients (20%) showed both endocapillary hypercellularity and crescent formation.[S5] Six (40%) patients in this group had also developed *de novo* DSA prior to biopsy, making ABMR an important differential diagnosis.

Twenty-one allograft biopsies without evidence of rejection, infection, or glomerulonephritis served as a negative control. In this "no rejection" group, 9 patients (43%) showed no major graft pathology, and 11 patients showed signs of CNI-toxicity (52%) as defined by severe arteriolar hyalinosis (aah2-3) or acute tubular cell injury, but no other major abnormalities indicating structural damage or inflammation.

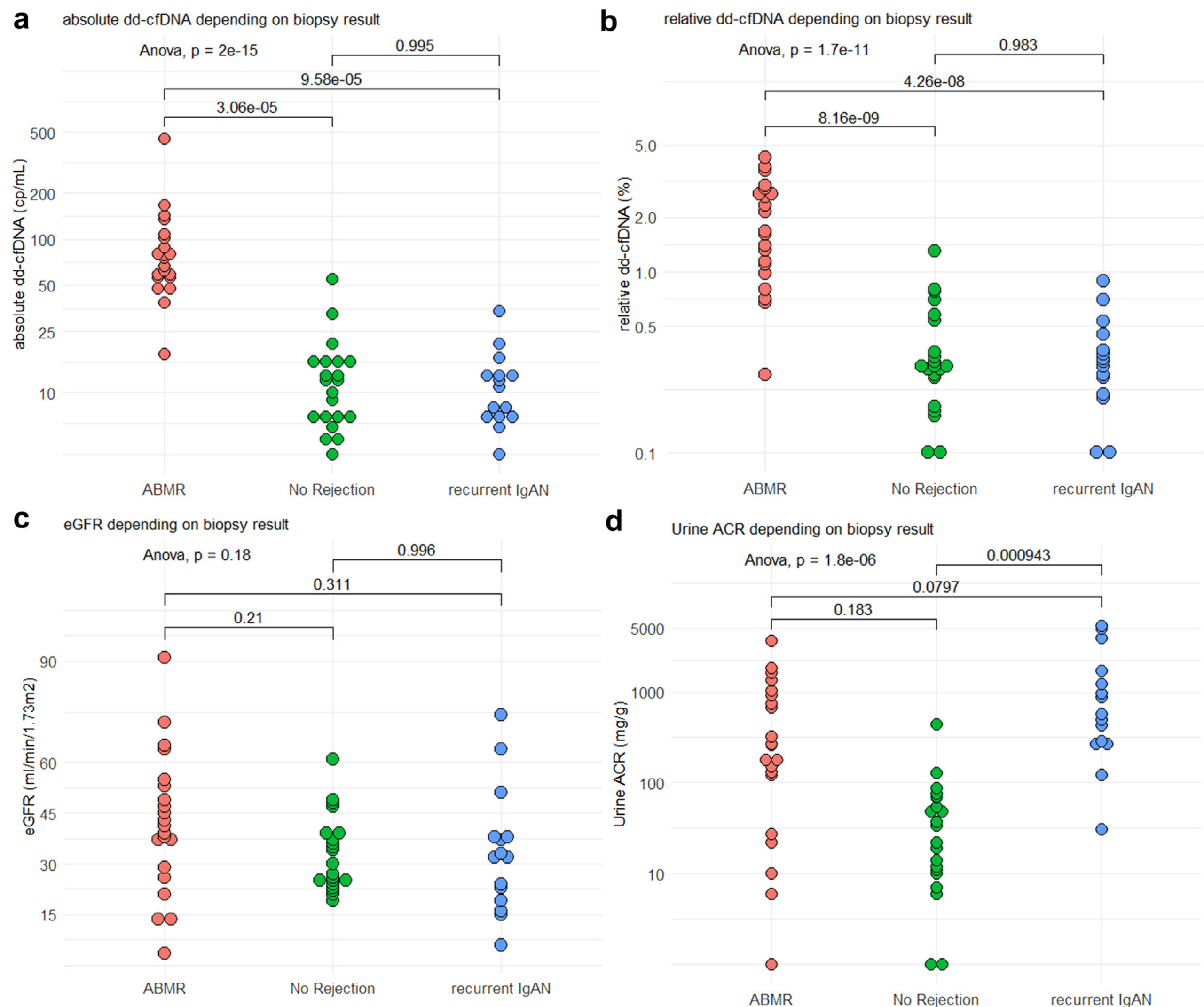

Figure 1. Dot plots showing biopsy-matched measurements of (a) absolute dd-cfDNA (copies/ml), (b) relative dd-cfDNA (%), (c) eGFR (ml/min per 1.73 m^2), and (d) urine albumin-to-creatinine ratio (mg/g) in kidney transplant recipients with antibody-mediated rejection, no rejection, and recurrent IgA nephropathy. eGFR, estimated glomerular filtration rate.

In this group, 1 patient had preformed DSA and 8 patients had *de novo* DSA.

Correlation of dd-cfDNA and Histopathology

Both absolute and relative dd-cfDNA were lower in patients with recurrent IgAN than in patients with ABMR (median 11 cp/ml, interquartile range [IQR] 7–13 vs. 76 cp/ml [IQR 57–103], $P < 0.001$; median 0.32% [IQR 0.24–0.41] vs. 1.68% [IQR 1.1–2.7], $P < 0.001$) but did not differ between patients with recurrent IgAN and no rejection (median 11 cp/ml [IQR 7–13] vs. 12 cp/ml [7–16], $P = 0.995$; median 0.32% [IQR 0.24–0.41] vs. 0.30% [IQR 0.26–0.54], $P = 0.983$) as shown in Figure 1. Using the previously established cutoff of 50 cp/ml for absolute quantification, no patient with recurrent IgAN, but 17 of 21 patients (81%) with ABMR had increased absolute dd-cfDNA levels. Using a cutoff of 0.5% for relative quantification, 3 of 15 patients (20%) with recurrent IgAN, and 20 of 21 patients (95%) with ABMR had increased relative dd-cfDNA levels. Interestingly, 4 patients (27%) with crescent formation as a sign of severe recurrent IgAN had low absolute (min–max: 6–21 cp/ml) and relative dd-cfDNA (min–max 0.26%–0.35%) as well.

Neither estimated glomerular filtration rate (median 32 ml/min per 1.73 m^2 [IQR 21–38] vs. 41.1 ml/min per 1.73 m^2 [IQR 29–53], $P = 0.31$) nor uACR (median 577 mg/g [IQR 277–1472] vs. median 259 [IQR 121–921], p=0.08) differed between patients with recurrent IgAN and ABMR. However, uACR was higher in patients with recurrent IgAN than in patients with no rejection (median 577 mg/g [IQR 277–1472] vs. median 34 mg/g [IQR 11–69], $P < 0.001$).

When performing ROC analysis to assess discrimination between recurrent IgAN and ABMR, we found that both absolute dd-cfDNA (area under the ROC

curve [AUC] 0.99 [95% CI: 0.96–1.00], and relative dd-cfDNA (AUC 0.94 [95% CI 0.87–1.00]) showed better discrimination than established biomarkers such as estimated glomerular filtration rate (AUC 0.66 [95% CI 0.49–0.84]), uACR (AUC 0.80 [95% CI 0.66–0.95]), and DSA (AUC 0.79 [95% CI 0.68–0.89]).

DISCUSSION

Despite continuous advances in kidney transplantation, there is further need to optimize long-term graft survival.[S6] Because there are a variety of causes for premature graft loss in general, and multiple factors contributing to its complexity in an individual patient, it is essential to improve monitoring of kidney allograft health.[9]

Routine measurements of estimated glomerular filtration rate, uACR, and DSA are of limited diagnostic value when distinguishing between different forms of graft injury in the late post-transplant phase. Therefore, allograft biopsy remains the gold standard for a definite diagnosis despite its known limitations, and is often a late diagnostic step limiting timely therapeutic intervention.

Recent research explored the role of dd-cfDNA to detect graft injury and aimed to evaluate its clinical validity and benefit. Although its diagnostic performance in alloimmune-mediated injury, especially in ABMR is well described, its potential to discriminate rejection from other patterns of graft inflammation, such as recurrent IgAN needs further investigation. This is of particular interest, because both ABMR and recurrent IgAN are important causes for premature graft failure and show comparable clinical findings.

To our knowledge, this is the first report focusing on the dynamics of dd-cfDNA in recurrent glomerular disease after transplantation. We demonstrate the correlation of dd-cfDNA with histologically-proven recurrence of IgAN compared to ABMR and no rejection. Our findings suggest that dd-cfDNA does not increase in recurrent IgAN even in cases of severe histological recurrence and clinical deterioration. Low dd-cfDNA levels could be explained by the slowly progressing nature of glomerular IgA immune complex deposition and the consecutive injury. Although the exact mechanisms of dd-cfDNA release in both entities still need clarification, we observed that dd-cfDNA increases already in the subclinical and potentially reversible stage of ABMR. Consequently, longitudinal measurement may identify patients with ABMR earlier than routine biomarkers and optimize biopsy timing, though its role in disease recurrence is not clear.

The limitations of this study are its small sample size, and the exclusion of potentially confounding diagnoses, both of which limit generalizability of AUC-ROC values into the clinical context.

We conclude that though dd-cfDNA-testing may help distinguish recurrent IgAN and ABMR noninvasively, kidney allograft biopsy will still be needed to distinguish recurrent IgAN from non-ABMR related pathologies in the presence of graft dysfunction, proteinuria, and normal dd-cfDNA levels.

DISCLOSURE

MO acts as a consultant to Oncocyte. JB, KB-K., and ES are employees of Chronix Biomedical GmbH, a subsidiary of Chronix Biomedical Inc. (an Oncocyte company), which holds intellectual property rights (EP 3004388B1, EP3201361B1 and US10570443B2).

FUNDING

Laboratory testing was sponsored by Oncocyte.

AUTHOR CONTRIBUTIONS

AA and BO conceived of the presented idea. AA, BO, KB, MC, and JK performed the investigation. JB and KB-K. performed the laboratory testing. AA, BO, and JB performed the data analysis. KB, MO, JB, and ES supervised the work. AA and BO wrote the manuscript. All authors have read and agreed to the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

Figure S1. Patient flow diagram.

Table S1. Inclusion and exclusion criteria.

Table S2. Dd-cfDNA results and renal function parameters of excluded biopsies.

STROBE Statement—checklist of items that should be included in reports of observational studies.

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