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Donor-derived Cell-free DNA in Infections in Kidney Transplant Recipients: Case Series

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Background. Donor-derived cell-free DNA (dd-cfDNA) is a noninvasive plasma biomarker to evaluate for transplant allograft rejection. The relationship between infectious complications in kidney allografts and dd-cfDNA has received cursory attention in prior publications. **Methods.** Retrospective review of all renal transplant recipients who underwent dd-cfDNA testing between November 2017 and August 2019. **Results.** We report on 7 cases in whom infections affecting the transplanted kidney were associated with elevation in dd-cfDNA without concomitant rejection or elevation in serum creatinine. Five patients had BK viremia, and 2 patients had urinary tract infection associated with elevated dd-cfDNA levels. **Conclusions.** These observations suggest that elevations in dd-cfDNA are not specific to kidney allograft rejection and can be associated with infections affecting the transplanted kidney. This biomarker may be valuable in evaluating infectious complications of kidney allografts.

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Various events including immunologic, vascular, structural, medication related, and infectious causes can affect kidney allograft function and longevity. Prompt diagnosis and treatment of these insults is essential and could improve the function and prolong the longevity of the graft.[1] Histological examination of an allograft biopsy is considered the gold standard for evaluation of abnormal kidney function to diagnose pathological processes, particularly various types of rejection.[2] Biopsy is an invasive procedure, carries a complication rate of about 1%, has associated logistic and scheduling burdens, and is resource intensive. Furthermore, variability in pathological diagnosis is common, and up to 25% of reports are nondiagnostic.[3,4] Therefore, other noninvasive methods to evaluate different forms of allograft injury are needed. Elevations in plasma donor-derived cell-free DNA (dd-cfDNA) have been described in the presence of graft rejection in liver, lung, heart, and kidney transplant recipients.[5-9]

Because organ injury prompts cell-free DNA (cfDNA) release, it is conceivable that trauma, infection, ischemia, or immune events may lead to cfDNA increases in the plasma. This is particularly relevant for transplantation where both rejection and infection are common. Moreira et al[10] described elevations in plasma and urinary cfDNA levels in the setting of infections, whereas Sigdel et al[11] described elevations in urinary dd-cfDNA in infections. We hypothesized that elevations in dd-cfDNA are not specific to rejection, and levels could be elevated in infections of kidney allografts. In this series, we report 7 cases of patients with graft injury causing elevations in dd-cfDNA during bacterial and viral kidney infections.

MATERIALS AND METHODS

After institutional review board approval, we performed a retrospective review of all kidney transplant recipients who underwent a dd-cfDNA testing (Allosure; Care Dx, Brisbane, CA) between November 2017 and August 2019 at our institution. All patients had the test for surveillance purposes; 28 patients were part of the Kidney Allograft Outcomes Allosure Registry. An abnormal dd-cfDNA result was defined as a value of ≥1%.[5] Patients with simultaneous

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dual-organ transplantation and those with a history of prior organ transplantation were excluded. All patients with at least 1 abnormal dd-cfDNA test and concomitant evidence of BK viremia, BK virus nephropathy (BKVN), or urinary tract infection (UTI) were included. BKVN was defined by the presence of viral cytopathic changes in the tubular epithelial cells and confirmed with positive immunohistochemical staining for SV40 large T antigen.[12] BK viremia was evaluated with quantitative polymerase chain reaction of the serum and reported as copies/mL. UTI was defined by the presence of symptoms and a positive urine bacterial culture with a count >100 000 colony-forming units. Donor characteristics evaluated included age, sex, living versus deceased donation, and kidney donor profile index. Recipient characteristics evaluated included age, sex, cause of end-stage kidney disease, type of dialysis, duration of dialysis, induction immunosuppressive regimen, and early graft function. Delayed graft function was defined as dialysis within 7 days after transplantation. Recipient serum creatinine level, microalbuminuria, kidney biopsy results, serial dd-cfDNA levels, BK viral load, and presence of donor-specific antibodies (DSAs) after transplant were examined.

RESULTS

During the study period, 392 patients had at least 1 dd-cfDNA test performed; 45 patients were excluded due to history of dual-organ transplantation or retransplantation. Twenty-nine patients had an elevated dd-cfDNA, whereas 318 had a dd-cfDNA value within normal limits. Out of the 29 patients with elevated dd-cfDNA, we identified 7 patients with elevated dd-cfDNA and concomitant evidence of infection affecting the kidney allograft: 5 patients had BK viremia, and 2 patients had bacterial UTI. Figures 1–7 illustrate the clinical course of each patient with elevated dd-cfDNA. Trends in creatinine, microalbuminuria, BK viremia, DSA, dd-cfDNA, and biopsy results are displayed for each patient. From the 318 patients with a nonelevated dd-cfDNA, 21 patients had evidence of an infection affecting the allograft: 17 with BK viremia and 4 patients with UTI. Table 1 shows the donor and the recipient characteristic for all 28 patients with infections. Overall, the test had a 25% (7/28) sensitivity to detect infection and specificity for infection of 24% (7/29). The predictive values for infection were 24.1% (7/22) positive predictive value and 93.4% (297/318) negative predictive value.

None of the 7 patients with elevated dd-cfDNA and infection had a concomitant increase in creatinine. In the recipients with infection but nonelevated dd-cfDNA, only 2 patients had elevation in their creatinine. The relationships between microalbuminuria and dd-cfDNA were variable. In case (number 6), the patient had microalbuminuria before UTI diagnosis likely to underlying antibody-mediated rejection. However, in case (number 7), microalbuminuria coincided with the diagnosis of UTI. In case number 5, the patient developed nephrotic range proteinuria with no identifiable explanation on the biopsy. In the 2 cases of UTI, elevation of dd-cfDNA occurred in close proximity to diagnosing the infection. In case number 6, the patient had baseline elevated dd-cfDNA to 1.8% from antibody-mediated rejection, but the level rose to 2.8% soon after a UTI was diagnosed. In case number 7, dd-cfDNA rose to 2% 12 days after diagnosing the infection. After UTI treatment, the dd-cfDNA trended down and normalized in both cases.

In the 5 cases of BK viremia, all patients had elevations in dd-cfDNA. Four patients had a kidney biopsy within a month of the elevated dd-cfDNA, and biopsies were negative for SV40 staining. One patient (number 2) had a kidney biopsy 6 months prior, which showed BKVN. The relationship between dd-cfDNA and the degree of BK viremia was variable; in case numbers 4 and 5, dd-cfDNA levels paralleled BK virus titers, whereas in case numbers 1–3, there was no correlation.

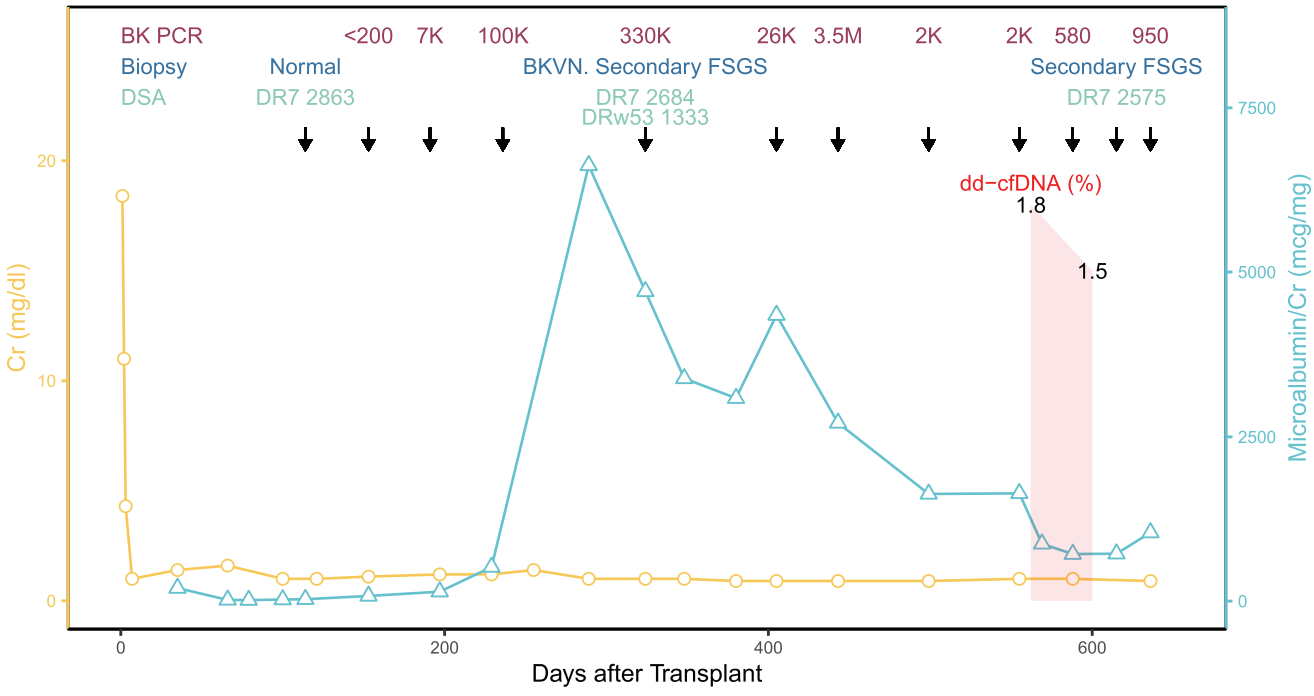

FIGURE 1. Case 1. Elevation in dd-cfDNA associated with BK viremia. BKV titers presented as number of virus copies/mL; DSA specificities presented as mean fluorescence index. BKVN, BK virus nephropathy; Cr, serum creatinine; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; FSGS, focal segmental glomerulosclerosis; PCR, polymerase chain reaction.

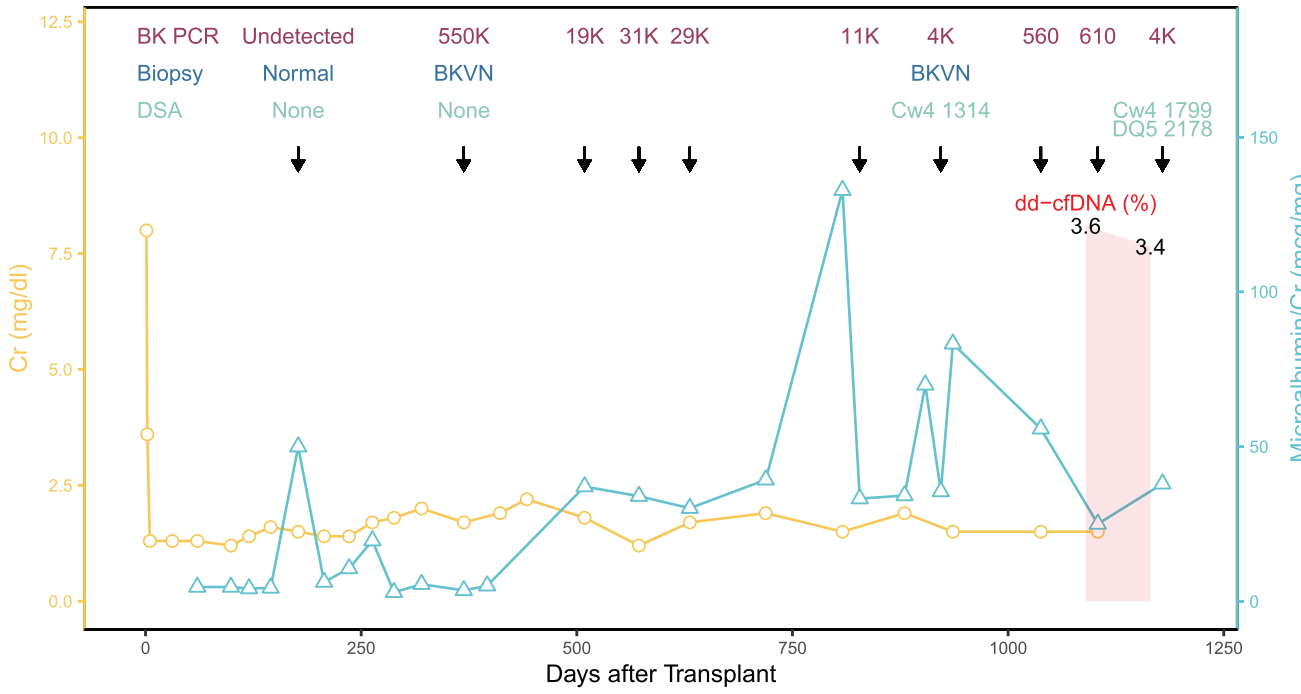

FIGURE 2. Case 2. Elevation in dd-cfDNA associated with BK viremia and BKVN. BKV titers presented as number of virus copies/mL; DSA specificities presented as mean fluorescence index. BKVN, BK virus nephropathy; Cr, serum creatinine; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody.

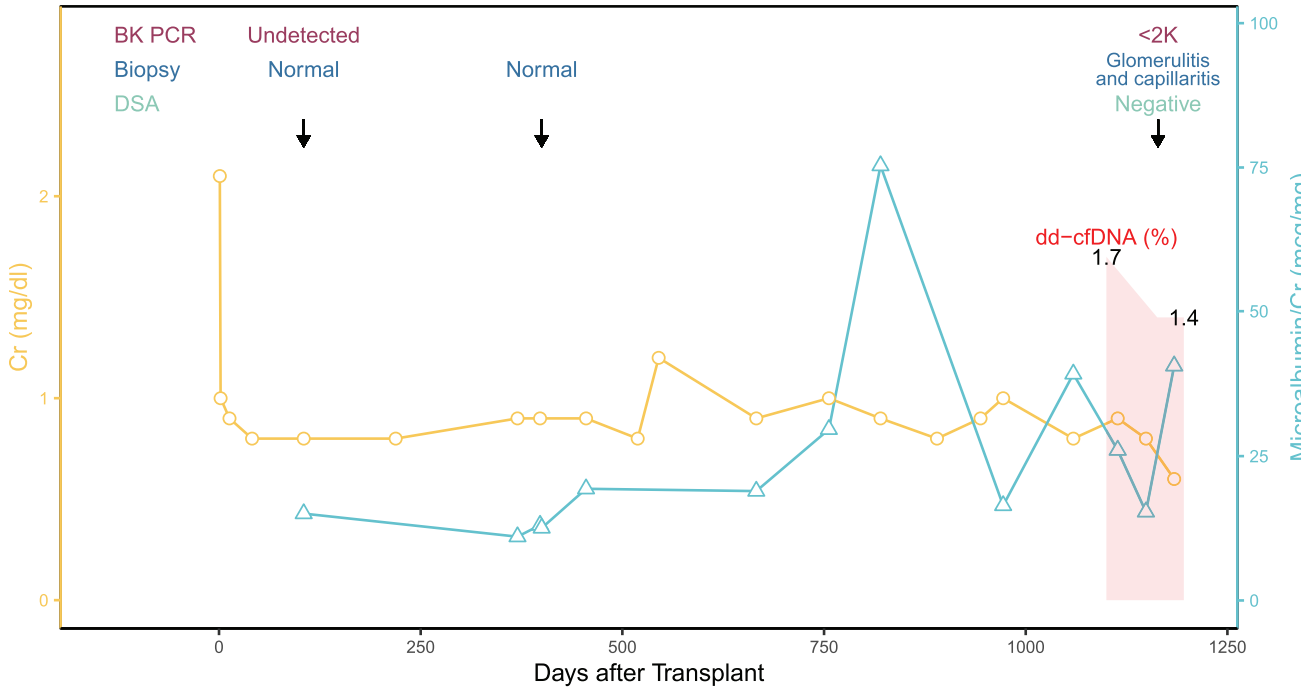

FIGURE 3. Case 3. Elevation in dd-cfDNA associated with BK viremia. BKV titers presented as number of virus copies/mL:, DSA specificities presented as mean fluorescence index. Cr, serum creatinine; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; PCR, polymerase chain reaction.

DISCUSSION

The use of dd-cfDNA became commercially available in October 2017 and has emerged as a promising and noninvasive biomarker to screen for the presence of allograft rejection. Elevations in dd-cfDNA often predate elevation in creatinine, making it valuable for detection of subclinical injury in transplants, potentially allowing for earlier interventions.[13,14] The use of dd-cfDNA to detect rejection has been reported in multiple studies with sensitivities ranging between 59% and 100%, specificities 72% and 85%, and areas under the curve

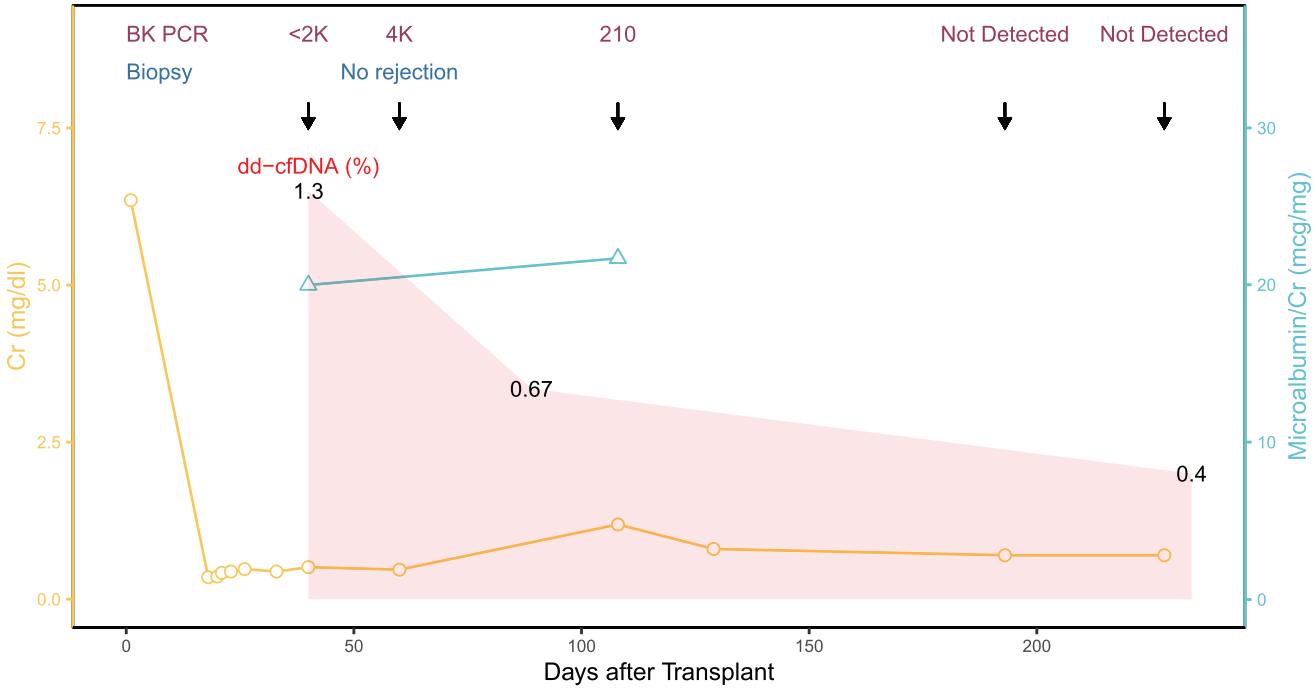

FIGURE 4. Case 4. Elevation in dd-cfDNA associated with BK viremia. BKV titers presented as number of virus copies/mL; DSA specificities presented as mean fluorescence index. Cr, serum creatinine; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; PCR, polymerase chain reaction.

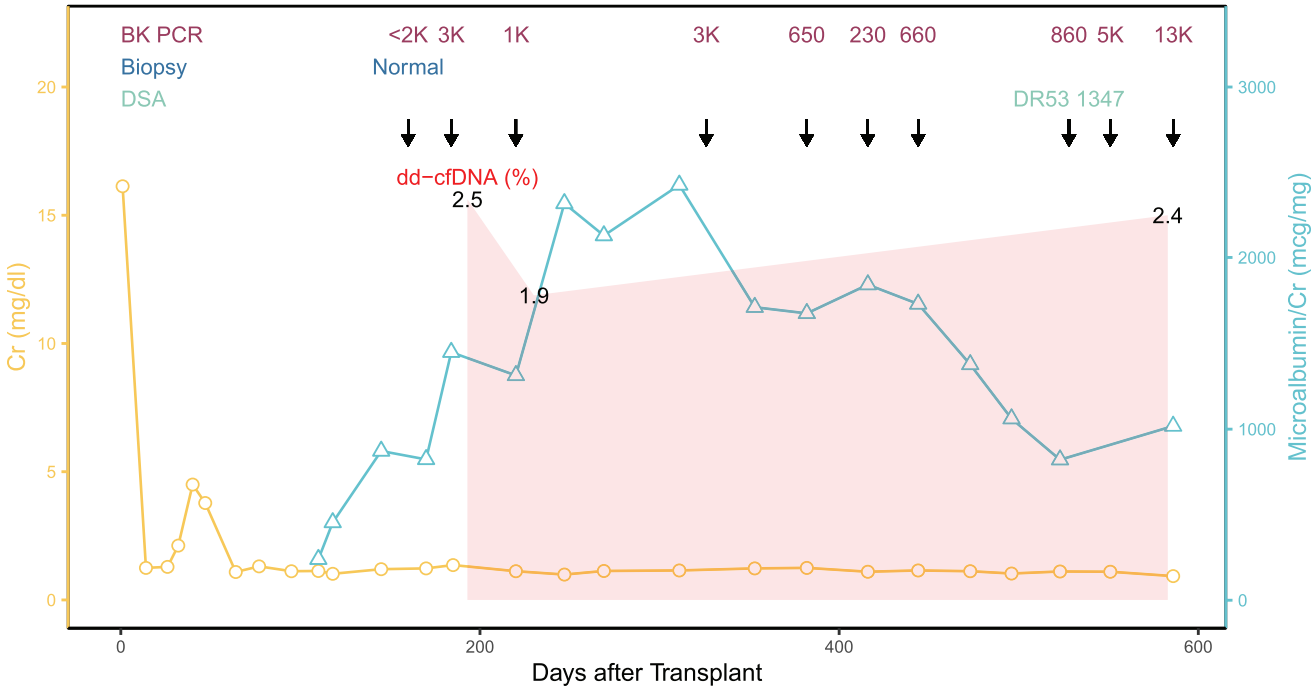

FIGURE 5. Case 5. Elevation in dd-cfDNA associated with BK viremia. BKV titers presented as number of virus copies/mL; DSA specificities presented as mean fluorescence index. Cr, serum creatinine; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; PCR, polymerase chain reaction.

of 0.74–0.82.[6,13,15] However, elevations in dd-cfDNA levels in the setting of infections affecting the kidney allograft have been limited to a few case reports.

Here, we report on 7 renal allograft recipients with elevation in plasma dd-cfDNA associated with infection: 5 cases of BK viremia and 2 cases of bacterial UTI. Our observations suggest that elevation in dd-cfDNA is likely attributable to graft injury from these infections. Six patients had a biopsy within 1 month of the elevated dd-cfDNA. In 3 cases, biopsy did not show injury in the graft. In 2 cases, biopsy findings were nonspecific and failed to explain dd-cfDNA elevation. One patient had recurrent antibody-mediated rejection with a biopsy showing capillaritis associated with multiple DSAs on multiple evaluations, but acute elevation of dd-cfDNA

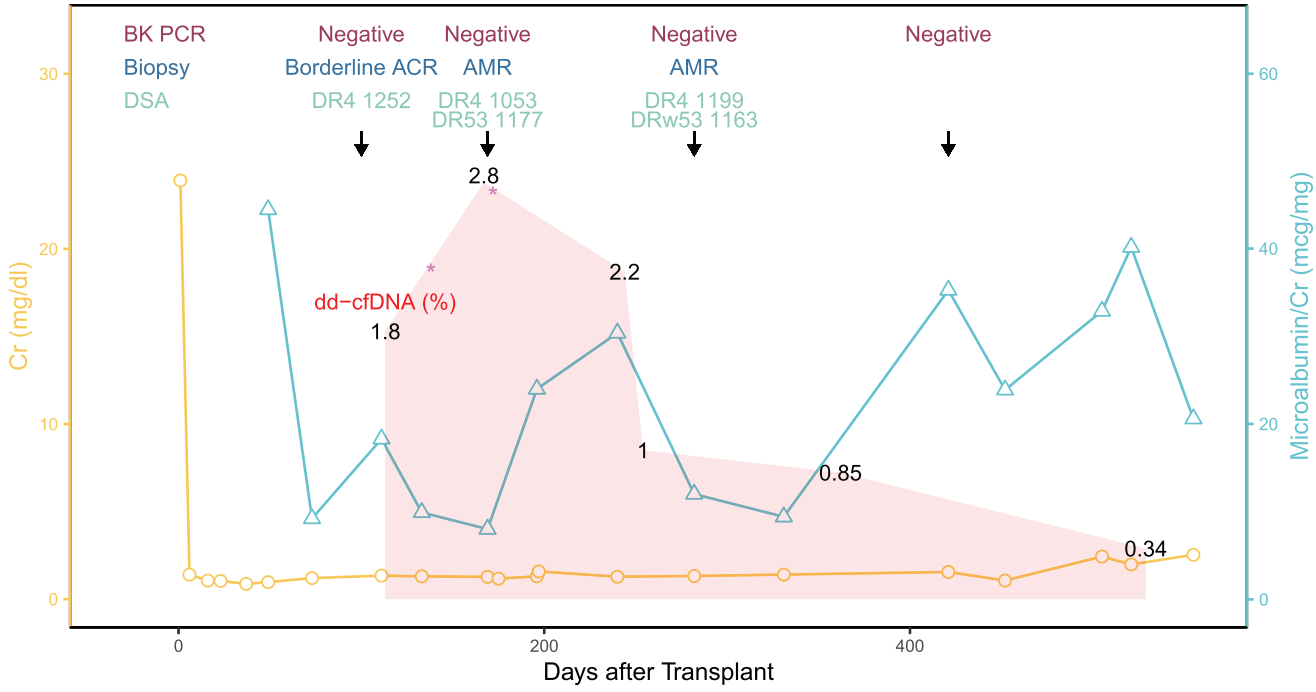

FIGURE 6. Case 6. Elevation in dd-cfDNA associated with recurrent urinary tract infections with *Enterobacter cloacae* complex and *Enterococcus faecalis* and antibody-mediated rejection (AMR). The asterisk indicates the occurrence of the urinary tract infection. BKV titers presented as number of virus copies/mL; DSA specificities presented as mean fluorescence index. Cr, serum creatinine; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; PCR, polymerase chain reaction.

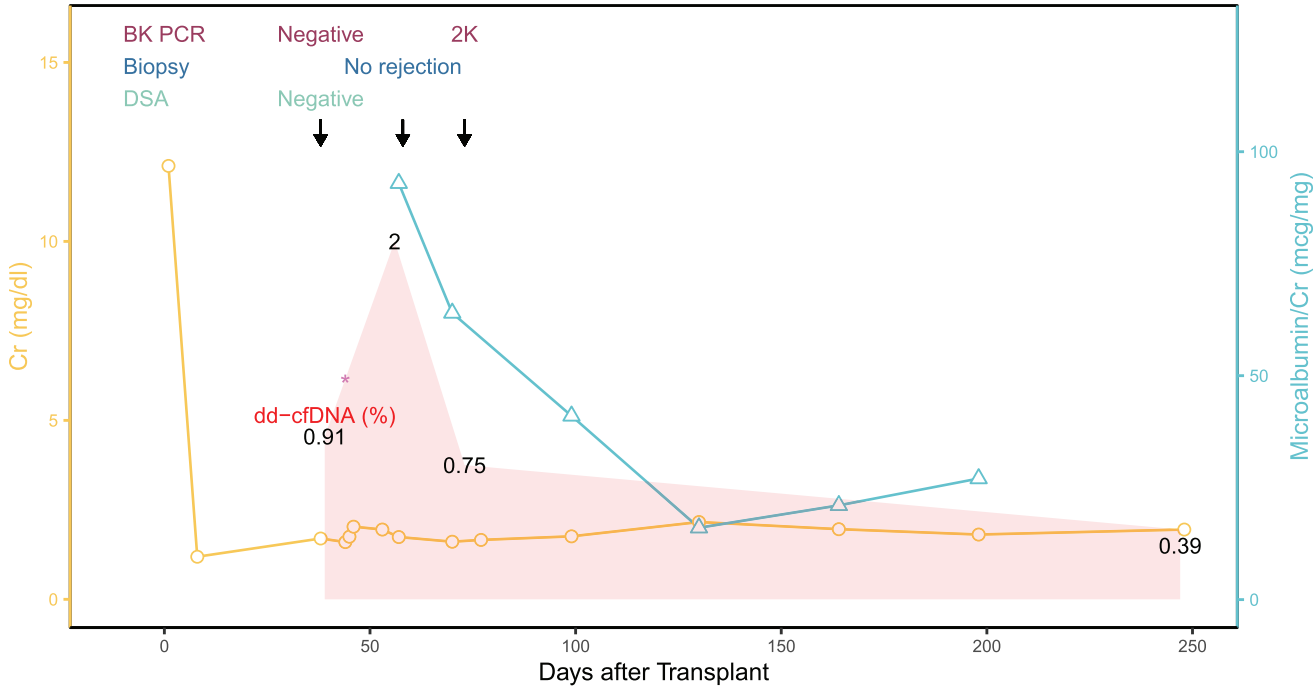

FIGURE 7. Case 7. Elevation in dd-cfDNA associated with urinary tract infection with *Acinetobacter baumannii* complex. The asterisk indicates the occurrence of the urinary tract infection. BKV titers presented as number of virus copies/mL; DSA specificities presented as mean fluorescence index. Cr, serum creatinine; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; PCR, polymerase chain reaction.

elevation coincided with recurrent UTI. With treatment and resolution of the infection, dd-cfDNA levels trended back to normal despite persistence of antibody-mediated rejection on biopsy. In one case, BKVN was diagnosed by biopsy 6 months before the elevated dd-cfDNA, but there was no biopsy closer to the dd-cfDNA result. This patient had persistent BK viremia with no additional clinical explanation for the elevated dd-cfDNA.

In all 7 cases, the subclinical injury identified by dd-cfDNA elevation was due to infections not captured by findings on

TABLE 1.

Donor and recipient characteristics

Case	Donor age (yrs)/sex	Living vs deceased DBD vs DCD	KDPI (%)	Recipient age (yrs)/sex	Cause of ESKD	Dialysis type	Years on dialysis	Induction	DGF vs IGF	Infection
1[a]	9/F	DBD	46	49/F	NSAID use	PD	12	Thymoglobulin	IGF	BK
2[a]	23/F	Living	NA	48/M	APKD	HD	1	Alemtuzumab	IGF	BK
3[a]	42/M	Living	NA	59/F	HTN/DM	HD	2	Thymoglobulin	IGF	BK
4[a]	31/M	Living	NA	32/F	SLE	HD	3	Alemtuzumab	IGF	BK
5[a]	8/F	DBD	44	56/F	SLE	PD	5	Alemtuzumab	IGF	BK
6[a]	28/M	DBD	18	36/F	FSGS	PD	2	Alemtuzumab	IGF	UTI
7[a]	55/F	DBD	84	62/M	HTN/DM	HD	3	Thymoglobulin	IGF	UTI
8	43/M	Living	NA	35/M	APKD	PD	0	Alemtuzumab	IGF	BK
9	42/F	Living	NA	38/M	Reflux nephropathy	HD	0	Alemtuzumab	IGF	BK
10	33/M	Living	NA	30/F	IgA nephropathy	HD	0	Alemtuzumab	IGF	BK
11	42/M	DBD	46	41/M	DM	HD	5	Alemtuzumab	DGF	BK
12	42/M	DBD	27	54/M	HTN/DM	HD	6	Alemtuzumab	DGF	BK
13	61/M	DBD	88	60/M	HTN	HD	6	Alemtuzumab	IGF	BK
14	63/F	Living	NA	60/M	APKD	Preemptive	0	Alemtuzumab	IGF	BK
15	76/M	DBD	99	74/M	HTN	HD	2	Basiliximab	IGF	BK
16	55/F	Living	NA	64/F	HTN	PD	1	Alemtuzumab	IGF	BK
17	54/M	DBD	90	65/F	HTN	PD	6	Basiliximab	IGF	BK
18	72/M	Living	NA	73/M	HTN	HD	1	Basiliximab	IGF	BK
19	36/M	DBD	50	59/M	HTN	HD	4	Thymoglobulin	IGF	BK
20	38/F	DBD	58	48/M	HTN/DM	HD	3	Thymoglobulin	DGF	BK
21	46/F	DBD	72	68/M	HTN	HD	8	Basiliximab	IGF	BK
22	33/F	DBD	26	38/M	HTN	HD	8	Thymoglobulin	DGF	BK
23	44/M	DBD	88	63/M	FSGS	HD	8	Alemtuzumab	DGF	BK
24	20/F	Living	NA	54/F	Alport syndrome	HD	0	Alemtuzumab	IGF	BK
25	67/F	Living	NA	73/M	IgA nephropathy	HD	0	Basiliximab	IGF	UTI
26	42/F	DBD	87	65/F	Bilateral nephrectomy	HD	8	Thymoglobulin	DGF	UTI
27	18/M	DCD	20	38/M	HTN	HD	10	Thymoglobulin	DGF	UTI
28	54/M	DCD	73	69/F	DM/HTN	Preemptive	0	Basiliximab	DGF	UTI

[a]Indicates the patients with elevated dd-cfDNA.

APKD, adult polycystic kidney disease; DBD, donation after brain death; DCD, donation after circulatory death; dd-cfDNA, donor-derived cell-free DNA; DGF, delayed graft function; DM, diabetes mellitus; ESKD, end-stage kidney disease; F, female; FSGS, focal segmental glomerulosclerosis; HD, hemodialysis; HTN, hypertension; IGF, immediate graft function; KDPI, kidney donor profile index; M, male; NSAID, non-steroidal anti inflammatory drugs; PD, peritoneal dialysis; SLE, systemic lupus erythematosus; UTI, urinary tract infection.

biopsy nor reflected by elevations in creatinine or microalbuminuria. These observations highlight the shortcomings of our current tools for detecting and diagnosing graft injury and function. In many pathological processes, the findings are focal and can be missed when evaluating biopsy specimens, whereas elevation in creatinine is a late finding that reflects substantial injury to the graft. Donor-derived cfDNA therefore can be regarded as a marker of injury not limited by the sampling error of a focal pathology affecting the organ. The interpretation of dd-cfDNA results in the setting of infections affecting the allograft must be done cautiously. In our cohort of the 28 patients with infections, 7 (25%) had elevated dd-cfDNA, suggesting either that the sensitivity is low or that most infections do not cause significant tissue damage. Further, of 29 patients with elevated dd-cfDNA, 7 (24%) had infection as the likely cause of the elevation, showing that there are multiple causes of tissue damage, including infection, and that this test identified tissue damage missed by other modalities. For the other 22 patients with elevated dd-cfDNA, the likely etiologies were rejection in 11, elevated DSA in 3, tacrolimus toxicity in 1, elevated dd-cfDNA after graft failure in 1, and there was no definitive diagnosis in 6 cases. For the 3 patients with elevated DSA, none had BK viremia, and 2 had biopsies that showed no rejection or other important abnormality, suggesting that dd-cfDNA may identify early antibody-mediated tissue injury. For the 6 with no definitive diagnosis, none had biopsies, 3 had no BK viremia, and 2 had no DSA, yet all had normal and stable renal function (Table 2).

Donor-derived cfDNA offers the advantage of being a noninvasive and perhaps a more sensitive biomarker for injury than creatinine or biopsy, but it lacks specificity regarding the type of injury. Because current dd-cfDNA detection techniques cannot differentiate one form of graft injury from another, we utilized dd-cfDNA as a surveillance test for injury of the graft. Elevated dd-cfDNA results prompt us to investigate different causes of injury, depending on the clinical scenario, by obtaining creatinine, urinary protein, urinalysis, relevant cultures, transplant renal ultrasound, allograft biopsy, serum DSA, and/or BKV polymerase chain reaction. Thus, in the setting of known BK viremia or the presence of UTI, elevated levels of dd-cfDNA reflect allograft injury even with a normal biopsy and creatinine. Elevated dd-cfDNA in a patient with known BK viremia could dictate the performance of a biopsy even if the creatinine and urinalysis are normal, whereas if the dd-cfDNA is normal, a biopsy may not be necessary. When evaluating recipients, the sensitivity and specificity of our current tools, such as clinical presentation, blood, and urinary studies, and imaging studies are each limited in their ability to

TABLE 2.

Patients with elevated dd-cfDNA and no infections

Patient	dd-cfDNA	Cr	Relation to baseline Cr	Kidney biopsy	DSA	BK PCR	Explanation for dd-cfDNA rise
1	1.1	1.5	Stable	AMR + BKVN	Detected	<1500	Rejection
2	3.9	2.1	Stable	AMR	Detected	Not detected	Rejection
3	2.9	1.3	Stable	AMR	Detected	Not detected	Rejection
4	2.8	1.2	Stable	AMR	Detected	Not detected	Rejection
5	1.1	1.6	Stable	AMR	Detected	Not detected	Rejection
6	1.9	1.3	Stable	AMR	Detected	Not detected	Rejection
7	1.8	2.8	Stable	AMR	Detected	Not detected	Rejection
8	1.5, 2.9	4.9	Stable	ACR	Not tested	<500	Rejection
9	1.3	1.6	Stable	ACR	Detected	Not detected	Rejection
10	1.8	3.0	Elevated	ACR	Not detected	Not detected	Rejection
11	1.2	3.4	Stable	ACR	Detected	Not detected	Rejection
12	1.3	1.0	Stable	Tubular injury	Not detected	Not detected	CNI toxicity
13	5.2	9.6	Elevated	No biopsy	Not tested	Not detected	Graft failure
14	1.5	1.2	Stable	Normal	Detected	Not detected	DSA
15	2.4	1.0	Stable	Normal	Detected	Not detected	DSA
16	1.7	1.5	Stable	Normal	Detected	Not detected	DSA
17	1.1, 1.2	1.0	Stable	No biopsy	Not detected	Not detected	Unknown
18	13	1.5	Stable	No biopsy	Not tested	Not tested	Unknown
19	1.5	1.7	Stable	No biopsy	Not tested	Not detected	Unknown
20	1.8	1.4	Stable	No biopsy	Not detected	Not detected	Unknown
21	1.3	1.3	Stable	No biopsy	Not tested	Not tested	Unknown
22	1.9	1.4	Stable	No biopsy	Not tested	Not tested	Unknown

ACR, acute cellular rejection; AMR, antibody-mediated rejection; BKVN, BK virus nephropathy; Cr, serum creatinine; CNI, calcineurin inhibitor; dd-cfDNA, donor-derived-cell free DNA; DSA, donor-specific antibody; PCR, polymerase chain reaction.

detect allograft injury. Thus, we propose adding dd-cfDNA to current algorithms to enhance our diagnostic sensitivity and accuracy in managing these patients.

Our study has several limitations. First, this is a small, retrospective study, making it difficult to prove causality. Second, the level of graft injury involved was not precisely defined, as the cases of UTI could have been cystitis with no graft involvement. In cases of BK virus infection, there is no quantitative direct correlation between the level of BK viremia and graft injury. Third, we used a dd-cfDNA threshold of 1% based on previous studies reporting on allograft rejection,[6] but this threshold is not definitive, and the association of allograft injury and dd-cfDNA is likely a continuous one rather than a strict categorical value.[16] Fourth, Oellerich et al[17] reported on the absolute value rather than the percentage of dd-cfDNA as being more discriminatory to detect graft rejection because the value would be independent of recipient-derived cfDNA. Because we used percentage to define graft injury, it is possible that increases in recipient cfDNA could diminish dd-cfDNA percentage, whereas a decrease in recipient cfDNA could increase the percentage of dd-cfDNA. These measurements could potentially explain the low sensitivity of the test for infection.

Elevations in plasma or urinary dd-cfDNA have been previously described during infections.[6,10,11] Ours is the first detailed case series reporting on elevations in dd-cfDNA during UTI and BK viremia affecting kidney allografts with in-depth analysis of the cases. Our report highlights that elevations in dd-cfDNA are not specific to rejection, but are observed in infections. We believe that this test will be valuable for surveilling and assessing kidney injury during infections, providing information about the degree of injury, a measure that will be useful for diagnosis, prognosis, and treatment. A future prospective trial is needed to better define the specificity and sensitivity of dd-cfDNA during infections, to correlate dd-cfDNA elevations with BK virus titers, to determine if these elevations signify a worse short- or long-term outcome for the graft, and to incorporate this test in a clinical diagnostic algorithm that also takes into account other tests and clinical metadata.

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