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Extremely High Cell-free DNA Levels Observed in Renal Allograft Patient With SARS-CoV-2 Infection

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Abstract. Beyond its widely recognized morbidity and mortality, coronavirus disease 2019 poses an additional health risk to renal allograft recipients. Detection and measurement of donor-derived cell-free DNA (dd-cfDNA), expressed as a fraction of the total cell-free DNA (cfDNA), has emerged as a noninvasive biomarker for allograft rejection. Here, we present a case report of a patient who was infected with severe acute respiratory syndrome coronavirus 2, 11 mo post–kidney transplant. The patient was serially monitored using an analytically and clinically validated massively multiplex PCR-based dd-cfDNA assay to assess allograft injury and risk for rejection. Over the course of infection, low dd-cfDNA fractions were observed (below the 1% cutoff) and were accompanied by unusually highly elevated levels of total cfDNA, which gradually declined as the infection resolved. The case study highlights the variability in total cfDNA levels during and after viral infection, and the need to consider both total and dd-cfDNA levels when clinically interpreting the results for allograft rejection. Furthermore, the study highlights the importance of serial testing, wherein an interplay between total cfDNA and dd-cfDNA can inform the optimization of a patient's immunosuppressive treatment regimen in response to infection.

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INTRODUCTION

Circulating cell-free DNA (cfDNA), arising from cellular death, is an established diagnostic marker with multiple clinical applications.[1] In individuals with organ transplantation, donor-derived cfDNA (dd-cfDNA) represents the fraction of total cfDNA that is released from an allograft of distinct genetic makeup. Immunologic responses toward an allograft promote cellular death,[2] resulting in the release of cfDNA, potentially increasing the dd-cfDNA fraction. Thus, dd-cfDNA has been established as a noninvasive biomarker for immunologic rejection of organ tissue.[3-5]

To prevent rejection, patients are initiated on life-long regimens of immunosuppressive medications following organ transplantation.[6] However, the dampened immune response may make these patients susceptible to infection.[7] The current coronavirus disease 2019 (COVID-19) pandemic, caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, poses a major health risk to

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

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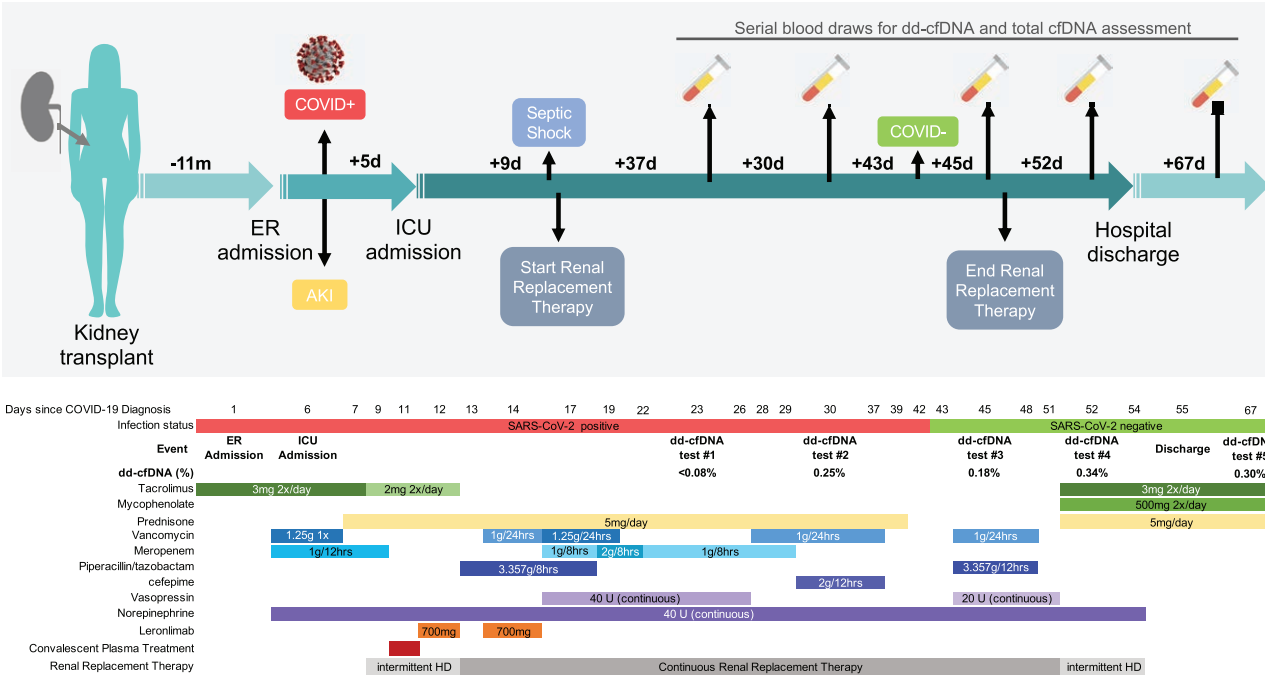

FIGURE 1. Clinical course of a kidney transplant patient with SARS-CoV-2 infection and associated medications. Top: schematic indicating key events during the clinical course of a kidney transplant patient with SARS-CoV-2. Bottom: medication strategy according to d of hospitalization. All timespoints stated are relative to the date of ER admission. AKI, acute kidney injury; COVID, coronavirus disease; dd-cfDNA, donor-derived cell-free DNA; ER, emergency room; ICU, intensive care unit; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

transplant recipients. Individuals with kidney transplants often have comorbidities that put them at risk for complications from COVID-19.[8,9] Acute kidney injury (AKI), for instance, has been observed in up to 42% of kidney transplant recipients with COVID-19.[10] As AKI and other respiratory viral infections, such as influenza, are associated with increased risk of allograft failure/rejection,[11,12] kidney transplant recipients with COVID-19 may also be at risk for graft rejection/failure. Thus, as our understanding of the potential risk factors and clinical manifestations of COVID-19 evolves, guidance on optimal disease management for transplant patients is urgently needed.[13] In clinical validity studies, a cutoff of ≥1% dd-cfDNA fraction has demonstrated up to 89% sensitivity for detecting active rejection (antibody-mediated rejection, T-cell mediated rejection) in patients with kidney transplants.[4] However, levels of total circulating cfDNA can vary significantly in various disease states and are affected by clinical and treatment-related factors.[14] Associations have also been demonstrated between cfDNA levels and severity of viral infections, including SARS-CoV-2.[15,16] Changes in total cfDNA levels can affect the calculated dd-cfDNA fractions, when the absolute concentration of dd-cfDNA remains constant,[17] which can potentially complicate the interpretation of dd-cfDNA test results. Here we present a case report of a kidney transplant recipient who contracted SARS-CoV-2 and was found to have extremely elevated levels of background cfDNA.

CASE PRESENTATION

A 50-y-old woman with end-stage renal disease, secondary to polycystic kidney disease, presented to the emergency room with

TABLE 1.
Clinical laboratory parameters

Laboratory parameters	Baseline (posttransplant)	Day 6 (ICU admission)	Day 23	Day 30	Day 41
White blood cell count ($10^3/\mu L$)	6.08	3.79	12.50	11.66	9.90
Lymphocyte count ($10^3/\mu L$)	0.45	0.10 (d 7)	0.93	1.22	1.38
Platelet count (per mm^3)	11.0	150	157	193.6	219
D-dimer (μg/mL)	N/A	3330	1863	7016	2049
C-reactive protein (mg/dL)	N/A	9.05	12.5	4.1	0.4
Procalcitonin (μg/mL)	N/A	3.19 (d 7)	0.87	1.12 (d 28)	0.28
Lactate dehydrogenase (U/L)	N/A	450	245	308	224
Serum creatine (mg/dL)	1.67	3.45	0.55	1.1	1.06
Estimated glomerular filtration rate (mL/min/1.73 m^2)	34	14	>89	56	58.5
Troponin (ng/L)	N/A	<0.04	<0.04 (d 24)	N/A	N/A
IL-6 (pg/mL)	N/A	<5	21	7	<2

ICU, intensive care unit.

a 4-d history of diffuse muscle pain in April 2020. The patient had received a deceased donor kidney transplant 11 mo prior (Figure 1). Following transplant, the patient had baseline creatinine levels between 1.4 and 1.6 mg/dL and was maintained on an immunosuppression regimen of prednisone 5 mg daily, tacrolimus 3 mg BID, and mycophenolate mofetil 1000 mg BID. Upon presentation, her initial muscle pains were ascribed to recent initiation of statin therapy and her medication was discontinued. Over the next 48 h, the patient developed bilateral leg swelling and shortness of breath. She had labored breathing with a respiratory rate of 16 breaths/min, oxygen saturation of 94% on room air, temperature of 101°F, and blood pressure of 99/67 mm Hg. A chest radiograph demonstrated bilateral infiltrates and an upper respiratory nasopharyngeal swab returned positive for SARS-CoV-2. Laboratory data were notable for lactate dehydrogenase (253 U/L), white blood cell count (WBC; 2.3×10^3/μL), and lymphocyte count (0.1×10^3/μL). Elevated serum creatinine 2.8 mg/dL indicated AKI at the time of admission.

The patient remained febrile for 3 d before developing acute respiratory distress requiring oxygen supplementation; her creatinine level increased to 3.5 mg/dL (Table 1). Because of worsening of her respiratory status, the patient was transferred to the intensive care unit (ICU) where she was intubated, put on a ventilator, and vancomycin, meropenem, and azithromycin were initiated for antibiotic coverage; mycophenolate mofetil was discontinued (Figure 1). At the time of ICU admission, the patient's lactate dehydrogenase (450 U/L), D-dimer (3330 μg/mL), IL-6 (<5 pg/mL), C-reactive protein (CRP; 9.05 mg/dL), and procalcitonin (3.19 μg/mL) levels were elevated above the normal ranges (Table 1). The patient rapidly progressed to septic shock requiring vasopressor therapy. Renal function continued to deteriorate with creatinine levels increasing to an average of 5 mg/dL on day 9, prompting initiation of renal replacement therapy (RRT). The patient was placed on intermittent hemodialysis and subsequently switched to continuous renal replacement therapy. The patient received convalescent plasma on day 11 and received the immune modulator, leronlimab on days 12 and 14, on an open-label compassionate-use basis.[18] With no significant improvement in her overall condition, tacrolimus was tapered until discontinuation on day 12 and her immunosuppression regimen included only prednisone at 5 mg/d

Because of the patient's AKI and reduction of immunosuppression, her dd-cfDNA fraction was monitored to detect allograft rejection using a massively multiplex PCR assay that analyzes genomic DNA and measures allele frequencies at >13 000 single nucleotide polymorphisms (Prospera; Natera Inc.).[4] Total cfDNA levels were determined by comparison to a reference standard using dimensionless units and were reported as multiples of median (MoMs), in which the median value of 500 units was determined from 150 sequential samples processed for dd-cfDNA testing (Prospera) in Natera's CLIA-certified laboratory. dd-cfDNA levels were reported as a percentage of the total cfDNA level. On day 23, the patient's

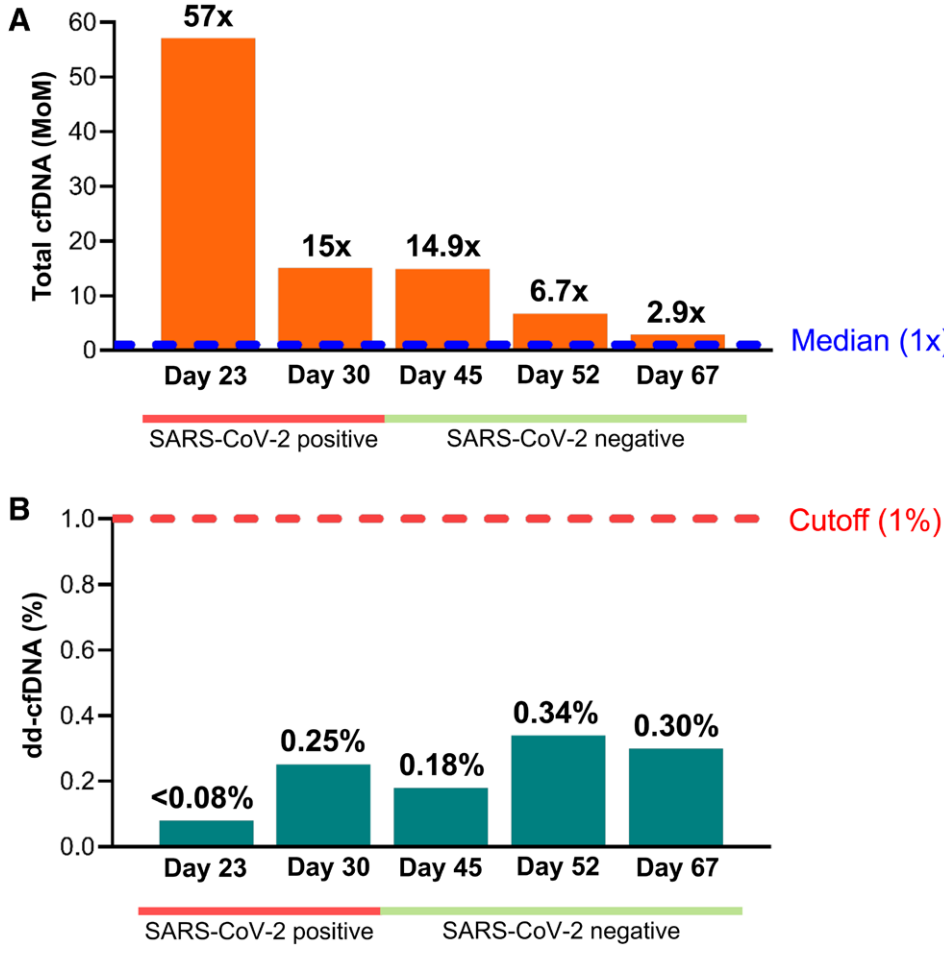

FIGURE 2. Comparative levels of dd-cfDNA and total cfDNA. Quantification of total cfDNA levels (top) and donor-derived cfDNA (dd-cfDNA; bottom) at each blood draw. Blue line indicates the median, to which total cfDNA levels are compared. Red line indicates the 1% dd-cfDNA cutoff for patients at risk of allograft rejection. cfDNA, cell-free DNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

dd-cfDNA fraction was <0.08% with a total cfDNA level at 57x MoM. A second blood draw on day 30 revealed a dd-cfDNA fraction of 0.25% with a total cfDNA level at 15x MoM (Figure 2). On day 23 the patient's IL-6 (21 pg/mL), CRP (12.5 mg/dL), and WBC (12.5×10^3/μL) levels had increased, whereas D-dimer (1863 μg/mL) and procalcitonin (0.87 μg/mL) levels decreased but remained elevated above the normal range. By day 30, the patient's IL-6 (7 pg/mL), CRP (4.1 mg/dL), and WBC (11.66×10^3/μL) levels had decreased but remained elevated above the normal range.

The patient first tested negative for SARS-CoV-2 on day 43. Three dd-cfDNA tests performed on days 45, 52, and 67, following clearance of the virus, reported dd-cfDNA fractions of 0.18%, 0.34%, and 0.30%, respectively, with corresponding total cfDNA levels of 14.9x MoM, 6.7x MoM, and 2.9x MoM (Figure 2). By day 51, the patient was weaned off vasopressors and was transitioned from continuous renal therapy to intermittent hemodialysis. She was reinitiated on tacrolimus, mycophenolate in addition to prednisone. The patient was discharged on day 55, with oxygen saturation of 100% on room air and off of RRT with a serum creatinine level of 1.35 mg/dL.

DISCUSSION

Kidney transplant recipients are at high risk of developing severe complications from SARS-CoV-2 infection,[10] including AKI, which is seen in a high proportion of COVID-19 patients.[19] Reduction in maintenance immunosuppression is recommended during severe life-threatening infections.[20] However, when AKI occurs in the setting of reduced immunosuppression, allograft injury from rejection or sepsis-related acute tubular necrosis is difficult to differentiate. Kidney biopsy, the gold standard to differentiate between the 2 diagnoses, often cannot be performed in individuals who are critically ill. Thus, the use of a noninvasive dd-cfDNA assay provides an alternative tool for assessing allograft rejection in this setting.

In the presented case, the initial dd-cfDNA fraction, determined during ongoing SARS-CoV-2 infection, did not indicate potential allograft rejection. However, this low fraction was associated with an unusually high total cfDNA level, which may have confounded the interpretation of dd-cfDNA results. Hemodialysis, hypertension, and septic shock can cause ischemic injury and release of cfDNA, contributing to overall increases in total cfDNA levels.[14,21,22] Thus, elevations in total cfDNA may be expected in this patient because of septic shock and RRT. However, fluctuations in total cfDNA and the corresponding dd-cfDNA levels across multiple tests in a relatively short period of time, highlight the need for serial testing. This may provide a cost-effective and noninvasive approach to inform treatment modulation as the patients recovers from the infection.

Multiple factors may have potentially contributed to the elevated cfDNA levels detected in this individual. In addition to tissue ischemia, viral infections, including SARS-CoV-2 have been shown to contribute to cfDNA release by multiple tissues, elevating overall cfDNA levels.[16,23] The initial elevation in total cfDNA, during SARS-CoV-2 infection, may be a result of cellular apoptosis resulting from inflammation or immunologic responses to the virus. Although the patient's total cfDNA levels decreased at subsequent timepoints, they remained elevated above the median threshold following resolution of SARS-CoV-2 infection. Thus, residual inflammation or unresolved tissue injury resulting from the patient's ongoing septic shock and RRT may have also contributed to the elevated cfDNA levels following clearance of the virus.

In the presented case, the decrease in total cfDNA levels over the course of multiple tests was accompanied by a gradual increase in dd-cfDNA fractions. This demonstrates how fluctuations in total cfDNA levels inherently affect the calculated dd-cfDNA fraction and may even mask the rise in dd-cfDNA fraction that could be indicative of graft injury or rejection. Recent studies have shown the utility of measuring absolute dd-cfDNA levels to mitigate such issues across a range of clinical presentations[17] and may even improve the ability to distinguish certain types of graft rejection.[24,25] Currently, commercially available tests do not incorporate absolute dd-cfDNA levels into assessments of rejection risk. Thus, consideration of total cfDNA levels in the interpretion of dd-cfDNA test results, could help inform patient management and the need to perform additional tests.

This case demonstrates the variability of total cfDNA during infection with SARS-CoV-2 and indicates the importance of serial dd-cfDNA testing to ensure accurate assessment of kidney allograft rejection in individuals with COVID-19. These findings suggest that considering total cfDNA levels can be useful in determining whether dd-cfDNA readings are representative of allograft state and in the management of a patient's immunosuppressive treatment regimen.

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