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Utility of Donor-Derived Cell-Free DNA in Detecting ABMR in Patients With AT1R Antibodies

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It is increasingly acknowledged that antibodies against human leukocyte antigens (HLAs) are implicated in less than 50% of histological features indicative of antibody-mediated rejection (ABMR).[1] During that past decade, non-HLA antibodies against angiotensin II type 1 receptor (AT1R) have been recognized to be associated with ABMR in the absence of donor-specific HLA antibodies (DSA).[2] The presence of AT1R antibodies is independently associated with high risk of episodes of ABMR and decreased long-term allograft survival.[3] The co-existence of negative DSAs and histological features of ABMR with pre-existing positive AT1R antibodies, underlies the need for noninvasive markers of rejection. We investigated the utility of donor-derived cell-free DNA (dd-cfDNA) in patients with positive AT1R antibodies and ABMR with no evidence of DSA.

We performed a multicenter retrospective analysis of patients with positive AT1R antibodies (One Lambda, Thermo Fisher, Waltham, Massachusetts) who had concomitant dd-cfDNA measurements (AlloSure, CareDx, Brisbane, California) for surveillance or worsening of allograft function concerning for rejection. These patients also underwent allograft biopsies demonstrating evidence of ABMR. A positive AT1R was defined as >10 IU/ml and dd-cfDNA >1%. Statistical analysis included spearman correlation curves and Student *t* tests using Stata 2019 (College Station, Texas).

RESULTS

We identified 16 kidney transplant recipients with histological features of ABMR with negative DSA and positive AT1R antibodies. Six (38%) were female, seven (44%) were Caucasian (44%), and seven (44%) were African American, with a mean age of 43 years at transplantation. Thirteen patients (81%) underwent deceased-donor kidney transplantation.

All patients had elevation of dd-cfDNA >1% before allograft biopsy with the exception of one (>0.5%), with median level of 2.6% (range, 0.66% to 7.9%). There was a trend towards an inverse association between levels of AT1R and dd-cfDNA (r = -0.2, $P = 0.2$), with stronger correlation for dd-cfDNA obtained for concern for rejection (r = - 0.5, $P = 0.12$) compared to those performed for all purposes (Figure 1). dd-cfDNA levels correlated well with Banff grades of rejection (glomerulitis [g] r = 0.3, $P = 0.12$; peritubular capillaritis [ptc] r = 0.4, $P = 0.05$; g + ptc r = 0.4, $P = 0.04$; interstitial inflammation [i] + tubulitis [t] r = 0.06, $P = 0.4$), with AT1R levels showing no correlation (Figure 2).

DISCUSSION

This study further shows the pathogenic role of AT1R antibodies in development of ABMR in the absence of DSA. Presence of dd-cfDNA > 1% has already been demonstrated to indicate higher probability of ABMR in kidney allografts and was elevated in all patients.[4] dd-cfDNA could be used for surveillance and detection of ABMR in patients with established AT1R antibodies, given lack of ability of AT1R antibody levels to predict incipient rejection and not all patients with these antibodies eventually

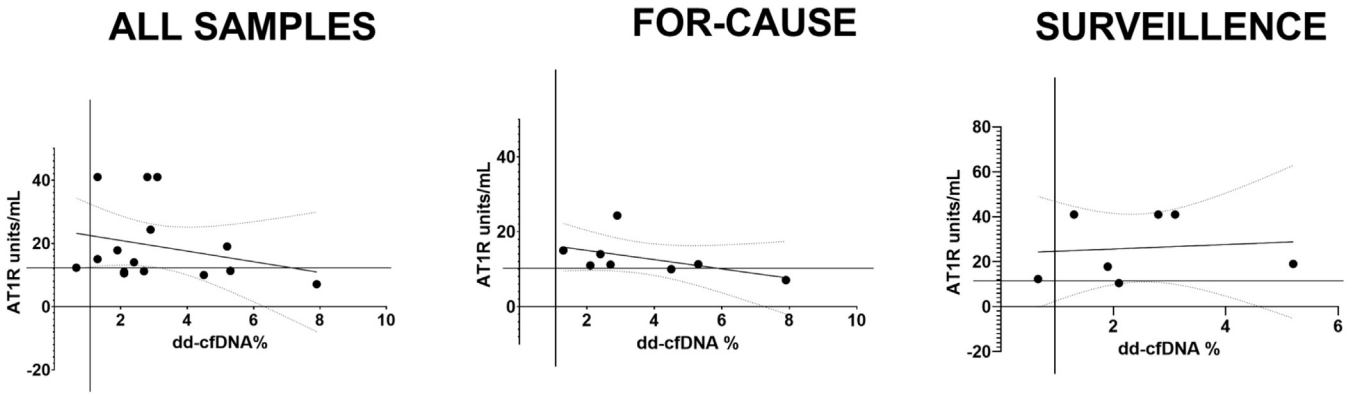

Figure 1. Spearman correlation curves for AT1R and dd-cfDNA levels for all samples, for-cause, and surveillance samples. AT1R, angiotensin 1 receptor antibodies; dd-cfDNA, donor-derived cell-free DNA.

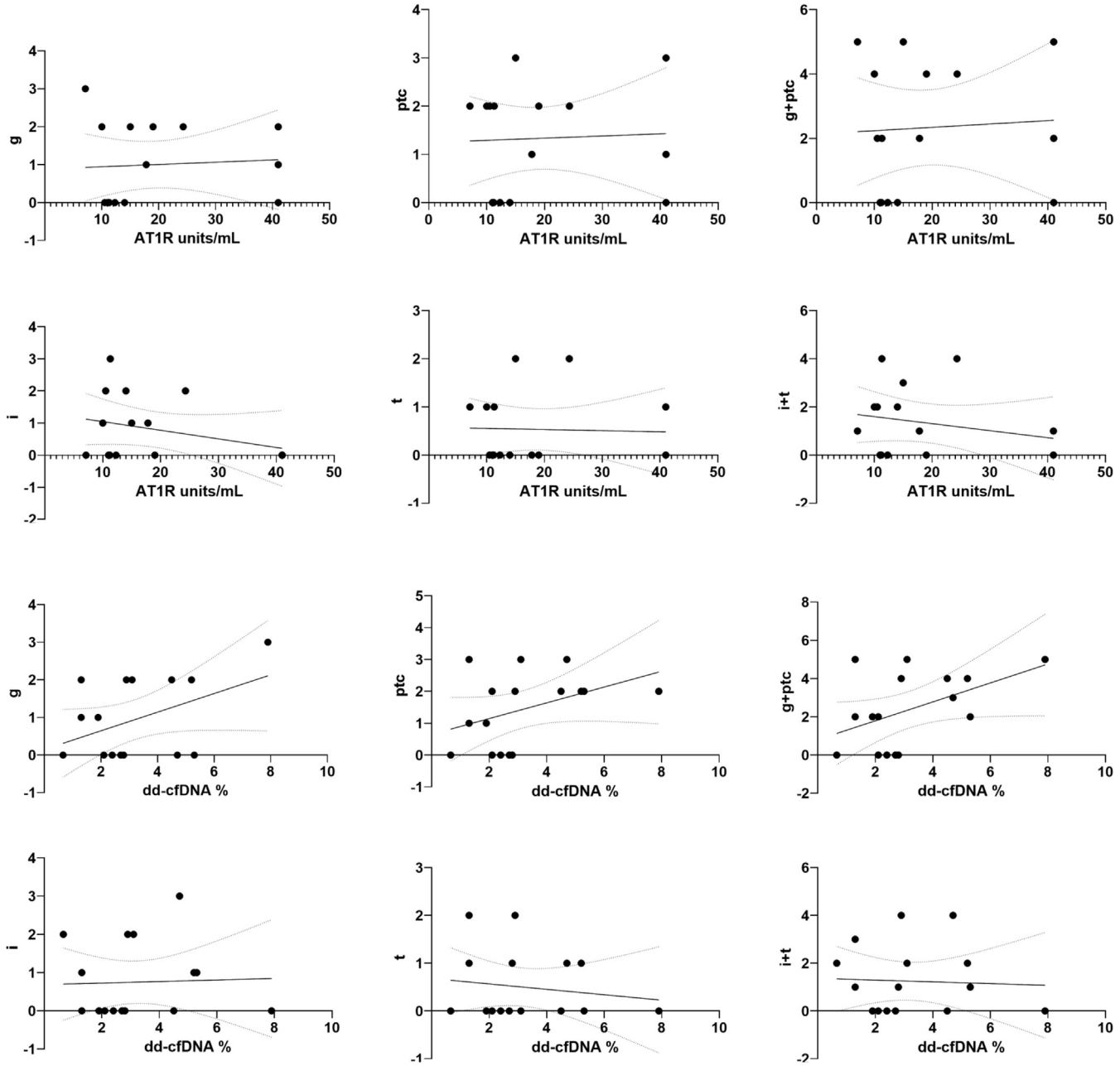

Figure 2. Spearman correlation curves for dd-cfDNA and AT1R levels for various Banff grades of rejection. AT1R, angiotensin 1 receptor antibodies; dd-cfDNA, donor-derived cell-free DNA; g, glomerulitis; I, interstitial inflammation; ptc, peritubular capillaritis; t, tubulitis.

experience rejection.[5] This is further highlighted by the finding that there was no significant correlation between AT1R antibodies and the histological features of ABMR and its severity. dd-cfDNA correlated well with the grades of g and ptc, which has been previously shown to be present in higher grades of rejection in patients with AT1R antibody associated acute rejection.[6] These findings are in line with a recent study in pediatric kidney transplants wherein dd-cfDNA was superior to AT1R antibodies in predicting presence of ABMR.[7] dd-cfDNA, therefore, has a utility in discriminating harmful from non-harmful presence of AT1R antibodies. In addition, in light of the aforementioned findings, dd-cfDNA may have utility in discerning pathogenicity of other non-HLA antibodies given increasing recognition of their contribution in mediating allograft injury.[8] Our study is limited by its small sample size and retrospective observational design. This study, however, is the first to assess correlation of AT1R antibody titers and dd-cfDNA, along with examining the utility of dd-cfDNA to predict presence of ABMR and its correlation with severity in the adult transplant population. Treatment would primarily include initially treating with angiotensin receptor blockers, with consideration for rituximab and plasmapheresis in the event of continued worsening kidney function despite treatment with angiotensin receptor blockers. With regard to monitoring, dd-cfDNA levels should be followed monthly for the first 3 months post-therapy to determine treatment efficacy and then quarterly to maintain surveillance for recurrence.

In conclusion, this study suggests that AT1R titers do not reflect severity of ABMR, whereas dd-cfDNA correlates well with severity grades of the Banff criteria. This study shows that dd-cfDNA could be used for monitoring and detecting rejection in patients with AT1R antibodies. Larger studies are needed to validate the findings of this study.

DISCLOSURE

IM, DK, and TA are on the advisory board for CareDx. TA declares consulting and speaker fees from CareDx. DCB has received personal fees from Amplyx, Allovir, CareDx, Natera, Sanofi, and Veloxis; has received research support from Amplyx, CareDx, and Natera; and has received honoraria from CareDx, Natera, and Sanofi. All the other authors have declared no competing interests.

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