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Donor-derived Cell-free DNA Combined With Histology Improves Prediction of Estimated Glomerular Filtration Rate Over Time in Kidney Transplant Recipients Compared With Histology Alone

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Background. Higher Banff inflammation and chronicity scores on kidney transplant biopsies are associated with poorer graft survival, although histology alone has limitations in predicting outcomes. We investigated if integrating donor-derived cell-free DNA (dd-cfDNA, Allosure; CareDx, Inc.) with Banff biopsy scores into a predictive model for estimated glomerular filtration rate over time can improve prognostic assessment versus histology alone. **Methods.** We identified 180 kidney transplant patients with dd-cfDNA assessed within 1 mo of biopsy. Using linear mixed–effects models, a prediction model of Banff histology scores and dd-cfDNA on estimated glomerular filtration rate over time was derived. Nested models were compared using the likelihood-ratio test, Akaike Information Criterion, and Bayesian Information Criterion to assess if inclusion of dd-cfDNA into a model consisting of Banff biopsy scores would improve model fit. **Results.** Univariate models identified significant covariate-by-time interactions for cg = 3 versus <3 (coefficient: −1.3 mL/min/1.73 m^2/mo; 95% confidence interval [CI], −2.4 to −0.2; $P = 0.02$) and ci + ct ≥ 3 versus <3 (coefficient: −0.7 mL/min/1.73 m^2/mo; 95% CI, −1.3 to −0.1; $P = 0.03$) and a trend toward significant covariate-by-time interaction for dd-cfDNA (coefficient: −0.5 mL/min/1.73 m^2/mo; 95% CI, −1.0 to 0.1; $P = 0.08$). Addition of acute inflammation (i, t, and v), microvascular inflammation (g and ptc), and inflammation in area of interstitial fibrosis and tubular atrophy scores to chronicity scores (cg ≥ 3 and ci + ct ≥ 3) did not improve model fit. However, a model including dd-cfDNA with cg and ci + ct with covariate-by-time interactions had a better model fit compared with cg and ci + ct alone (likelihood-ratio test statistic = 21.1; df = 2; $P < 0.001$). **Conclusions.** Addition of dd-cfDNA to Banff biopsy scores provided better prognostic assessment over biopsy characteristics alone.

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INTRODUCTION

The Banff classification of rejection in kidney transplantation relies on histologic assessment interpreted in the presence or absence of donor-specific antibodies (DSAs).[1] Recognizing that rejection can be present in a functionally stable allograft, measures of allograft function, such as creatinine, glomerular filtration rate (GFR), or proteinuria, are not included in the Banff criteria for rejection. Given this, it is not surprising

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that allografts with similar histologic appearances can have divergent prognoses and that integration of functional and immunologic parameters with histology predicts outcomes better than histology alone.[2] Prior studies have questioned the specificity of the Banff classification system and argued that histologic patterns of rejection might reflect nonimmune mediated injury in some circumstances.[3] The Banff working group has considered associations with graft outcome when assessing the significance of key histologic features of rejection, and it could be argued that lesions associated with long-term stability in graft function might not be representative of immune-mediated injury.[4] Therefore, the diagnostic classification of rejection might be enhanced by including determinants of allograft function and prognosis, which are best assessed using prognostic models.

Donor-derived cell-free DNA (dd-cfDNA, Allosure; CareDx, Inc.), a biomarker that can detect allograft injury, recently gained Medicare approval in 2017 for the assessment of rejection in adult kidney transplant recipients. Validation studies have indicated that higher percentages of dd-cfDNA in the blood correlate with the presence of rejection identified on biopsy,[5,6] with recent literature suggesting that dd-cfDNA might have some prognostic capability.[7] In this study, we investigated whether dd-cfDNA can predict the subsequent trajectory of estimated GFR (eGFR) and whether the combination of dd-cfDNA with histology can better inform on allograft prognosis than histology alone.

MATERIALS AND METHODS

This study was approved by the Cedars-Sinai Medical Center Institutional Review Board (Pro00020945). The study design, data analysis, and writing of the manuscript was performed by the authors alone and was not supported by any outside entity. Beginning in August, 2017, a total of 180 unique kidney transplant patients who were at least 1 mo posttransplant and followed at Cedars-Sinai Medical Center had assessment of dd-cfDNA within 1 mo of kidney transplant biopsy and were included in the study. All patients had dd-cfDNA assessment per manufacturer guidelines, with the following considered exclusion criteria: (1) age <18 y old, (2) pregnant status, (3) recipient of a transplant from an identical twin, (4) recipient of a nonkidney solid organ transplant, and (5) prior recipient of an allogeneic bone marrow transplant. In our center, dd-cfDNA and biopsies are assessed "for-cause" to investigate a suspicion of rejection in the setting of allograft dysfunction or presence of DSA. Biopsies were graded according to Banff 2019 criteria, which establish a minimum threshold for interstitial inflammation involving 10%–25% of unscarred cortical parenchyma (i1 lesion) in the presence of tubulitis as criteria for borderline cell-mediated rejection (CMR).[8] Because there was no difference in dd-cfDNA values between borderline CMR and CMR cases, histology meeting criteria for borderline CMR was classified as CMR for this study.

All serum creatinine values were recorded beginning with the most recent assessment before dd-cfDNA measurement until graft loss (n = 5), death (n = 2), or the end of the follow-up period on November 14, 2019. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation.[9] Linear mixed effects models using random slopes and intercepts and an unstructured covariance matrix were then fitted to derive a prediction model for eGFR over time. The fixed effect represents the average rate of change in eGFR by treatment group, whereas the random effect accounts for subject-specific correlation between repeated measures of eGFR within an individual. Univariate predictors and their interactions with time were tested in the model and included: recipient characteristics (age at dd-cfDNA assessment, gender, donor type [deceased versus living], presence of DSA), Banff biopsy scores (cg, ci + ct, g + ptc, inflammation in area of interstitial fibrosis and tubular atrophy [i-IFTA], i + t, and v-scores), and dd-cfDNA values (dichotomized into upper 2 quartiles versus lower 2 quartiles).

Five-fold crossvalidation was performed to identify the best-fitting model. For this procedure, the study population was randomly split into 5 equal-sized groups, and the model selection procedure was applied separately on 5 training sets incorporating a unique combination of 4 of the 5 folds. The best-fitting model identified from the training sets was then fitted on each remaining holdout (validation) set not utilized in the model training procedure. Covariates with a covariate-by-time interaction $P < 0.05$ on univariate analysis were entered into a multivariable linear mixed effects model. Nested models were compared using the likelihood-ratio (LR) test, Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC) to assess if inclusion of dd-cfDNA into a parsimonious model consisting of Banff biopsy scores would improve model fit. The LR test is a chi-squared test that assesses whether a model maximizes the likelihood function over another, whereas the AIC and BIC are methods that balance goodness of fit and the complexity of the model. Both the AIC and BIC favor the simplest model that yields the best fit, with a lower AIC and BIC indicating better goodness-of-fit.

A sensitivity analysis was performed on a subgroup of the study population excluding ABO-incompatible recipients and recipients with BK nephropathy, given that biopsies from both types of recipients have histologic features that overlap with but are not necessarily indicative of rejection (C4d-positivity in ABO-incompatible recipients and tubulointerstitial inflammation in BK nephropathy).

All P values were 2-tailed and a $P < 0.05$ was considered statistically significant. All analyses were performed using Stata version 14 (StataCorp; College Station, TX).

RESULTS

Table 1 describes the baseline characteristics of the study population. Assessment of dd-cfDNA was performed at a median of 659 d posttransplant (interquartile range [IQR], 81-2113). The majority of patients included were recipients of a deceased donor kidney. Fifty-seven patients (32% of the study population) had DSA at the time of dd-cfDNA assessment, with the vast majority consisting of class II DSA (88%). There was balanced representation of CMR, antibody-mediated rejection (AMR), and mixed CMR/AMR diagnoses.

The median dd-cfDNA in the study population was 0.70% (IQR, 0.28%–1.6%). Figure 1 compares the distribution of dd-cfDNA values corresponding to 4 clinical diagnostic categories: (a) no rejection, (b) isolated CMR, (c) isolated AMR, and (d) mixed CMR/AMR. There were significant differences in the distribution of dd-cfDNA between the 4 groups ($P < 0.001$). Pairwise comparisons indicated that the distribution of dd-cfDNA was higher among patients with isolated CMR (median, 0.80%; IQR, 0.33%–2.5%; $P < 0.001$), isolated AMR (median, 1.4%; IQR, 1.1%–2.7%; $P < 0.001$), and CMR/AMR (median,

TABLE 1.

Baseline characteristics

Recipient characteristics	n = 180
Age at dd-cfDNA assessment, mean ± SD	50 ± 14 y
D posttransplant at assessment, median (IQR)	659 (81–2113)
Male (%)	118 (66)
Donor type (%)	
Deceased	124 (69)
Living	56 (31)
Donor-specific antibodies (%)	57 (32)
Class I only	7 (4)
Class II only	44 (24)
Class I and class II	6 (3)
MFI, immunodominant DSA; median (IQR)[a]	10 000 (6250–17 500)
eGFR at dd-cfDNA assessment, mean ± SD	45 ± 21 mL/min/1.73 m^2
Donor-derived cell-free DNA %, median (IQR)	0.70% (0.28%–1.6%)
Quartile 1–2 (range)	0.15%–0.68%
Quartile 3–4 (range)	0.71%–16%
Biopsy characteristics (%)	
No rejection	84 (47)
Isolated cell–mediated rejection	45 (25)
Isolated antibody–mediated rejection	27 (15)
Mixed cell–mediated/antibody-mediated rejection	24 (13)

[a]Among 57 patients with DSA at the time of dd-cfDNA assessment.
dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MFI, mean fluorescence intensity.

1.5%; IQR, 0.84%–2.6%; $P < 0.001$) compared with those with no rejection (median, 0.35%; IQR, 0.19%–0.60%). The distribution of dd-cfDNA was higher among patients with isolated AMR ($P = 0.01$) and CMR/AMR ($P = 0.04$) compared with isolated CMR. There was no difference in the distribution of dd-cfDNA between patients with isolated AMR and those with mixed CMR/AMR ($P = 0.58$). With regard to cases of isolated CMR, there was no difference in dd-cfDNA between cases of borderline CMR (n = 21; median, 0.80%; IQR, 0.35%–1.3%) and cases of isolated CMR 1A or above (n = 24; median, 0.76; IQR, 0.29%–4.4%; $P = 0.39$).

Table 2 shows the distribution of Banff histologic scores corresponding to the 4 clinical diagnostic categories of no rejection, isolated CMR, isolated AMR, and mixed CMR/AMR. There was 1 patient classified as having no rejection despite having an isolated v-lesion (v1). This patient presented with a transiently increased creatinine and a dd-cfDNA value of 1.6%. There were no other histologic features of CMR or AMR identified on biopsy. On subsequent testing, the patient's creatinine spontaneously returned to baseline and clinical suspicion of rejection was low. The patient was not treated for rejection and was thus assigned a clinical diagnosis of no rejection. Ten patients had a cg-score ≥1a, were C4d-negative, and did not have current or prior DSA, thus not meeting criteria for chronic AMR. Additionally, there were 9 recipients of an ABO-incompatible allograft found to have C4d ≥ 2 without concomitant glomerulitis, peritubular capillaritis, or DSA and were classified as not having AMR. As expected, there was a preponderance of tubulointerstitial inflammation observed among patients with CMR, whereas microvascular inflammation (glomerulitis and peritubular capillaritis) and glomerular changes (transplant glomerulopathy) were more commonly seen among patients with AMR.

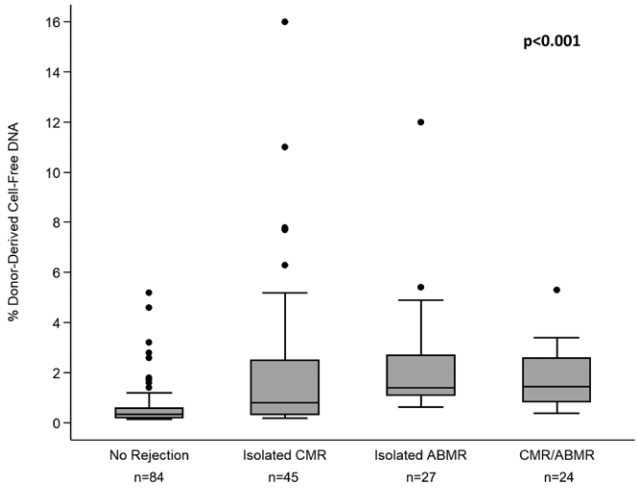

FIGURE 1. Box and whiskers plot comparing the distribution of donor-derived cell-free DNA % across histologic diagnoses. ABMR, antibody-mediated rejection; CMR, cell-mediated rejection.

Figure 2 shows the eGFR trajectory over time corresponding to each individual study participant. Linear mixed effects models were fitted to model the association of recipient characteristics, Banff histologic scores, and dd-cfDNA on eGFR over time. Model discovery was performed using 5-fold cross-validation and observed that the best-fitting model consisted of a combination of the Banff biopsy scores of cg (cg = 3 versus cg < 3) and ci + ct (≥3 versus <3) with dd-cfDNA (upper 2 quartiles versus lower 2 quartiles). This model yielded a mean root mean square error of 7.6 mL/min/1.73 m^2 (SE: 0.3 mL/min/1.73 m^2) across the 5 validation sets. Model output applied to the entire dataset is shown in Table 3, and measures of model fit are shown in Table 4. Table 3a shows the output for univariate linear mixed effects models. In the table, the reference coefficient represents the slope in eGFR per month (Δ eGFR/mo), whereas the coefficient for the interaction represents the difference in eGFR slope between the reference and comparator groups. Δ eGFR/mo for the comparator group equals the sum of the coefficients for the reference group and the interaction. There was no association between recipient or donor characteristics and Δ eGFR/mo on univariate analysis. The covariate-by-time interactions for cg and ci + ct were significant, indicating that biopsies with higher chronicity scores were associated with a steeper decline in eGFR over time compared with biopsies with milder degrees of chronicity. There was a trend toward significance for the interaction between dd-cfDNA and time (coefficient –0.5; 95% CI, –1.0–0.05; $P = 0.08$), providing a signal that higher percentages of dd-cfDNA may be associated with eGFR decline. Covariate-by-time interactions for i + t and i-IFTA were also borderline significant. Table 3b shows the multivariable model output for cg and ci + ct together with their associated covariate-by-time interactions, again indicating that biopsies with higher chronicity scores were associated with a steeper decline in eGFR over time compared with biopsies with less chronicity. Table 3c shows the regression output for Banff chronicity characteristics with dd-cfDNA values together. Inferences from this model paralleled those observed from Tables 3a and b.

Table 4 shows measures of model fit for the nested multivariable models integrating Banff chronicity scores (cg and ci + ct) with dd-cfDNA and their corresponding covariate-by-time interactions versus cg and ci + ct with covariate-by-time interactions. The LR test was significant ($P < 0.001$), indicating that the more complex model including biopsy scores with

TABLE 2.

Distribution of Banff histology scores by clinical diagnosis

Banff score	No rejection (n = 84)	Isolated CMR (n = 45)	Isolated AMR (n = 27)	Mixed CMR/AMR (n = 24)	*P*
i (%)					<0.001
0	77 (92)	3 (7)[a]	26 (96)	1 (4)	
1	1 (1)	19 (42)	1 (4)	10 (42)	
2	4 (5)	11 (24)	0 (0)	6 (25)	
3	2 (2)	12 (27)	0 (0)	7 (29)	
t (%)					<0.001
0	46 (55)	1 (2)	14 (52)	0 (0)	
1	32 (38)	13 (29)	12 (44)	12 (50)	
2	3 (4)	15 (33)	1 (4)	6 (25)	
3	3 (4)	16 (36)	0 (0)	6 (25)	
v (%)					0.003
0	83 (99)	36 (80)	27 (100)	22 (92)	
1	1 (1)	7 (16)	0 (0)	2 (8)	
2	0 (0)	2 (4)	0 (0)	0 (0)	
3	0 (0)	0 (0)	0 (0)	0 (0)	
ci (%)					0.02
0	40 (48)	13 (29)	19 (70)	11 (46)	
1	28 (33)	14 (31)	5 (19)	7 (29)	
2	14 (17)	11 (24)	2 (7)	5 (21)	
3	2 (2)	7 (16)	1 (4)	1 (4)	
ct (%)					0.07
0	42 (50)	14 (31)	19 (70)	11 (46)	
1	26 (31)	14 (31)	5 (19)	7 (29)	
2	14 (17)	11 (24)	2 (7)	4 (17)	
3	2 (2)	6 (13)	1 (4)	2 (8)	
g (%)					<0.001
0	79 (94)	37 (82)	7 (26)	6 (25)	
1	2 (2)	6 (13)	9 (33)	13 (54)	
2	3 (4)	2 (4)	7 (26)	4 (17)	
3	0 (0)	0 (0)	4 (15)	1 (4)	
ptc (%)					<0.001
0	73 (87)	24 (53)	5 (19)	4 (17)	
1	2 (2)	7 (16)	4 (15)	1 (4)	
2	7 (8)	11 (24)	11 (41)	6 (25)	
3	2 (2)	3 (7)	7 (26)	13 (54)	
C4d (%)[b]					<0.001
0	78 (93)	41 (91)	12 (44)	8 (33)	
1	1 (1)	0 (0)	1 (4)	4 (17)	
2[c]	0 (0)	1 (2)	7 (26)	3 (13)	
3[c]	5 (6)	3 (7)	7 (26)	9 (38)	
cg (%)					<0.001
0	78 (93)	41 (91)	11 (41)	12 (50)	
1a	3 (4)	1 (2)	6 (22)	4 (17)	
1b	2 (2)	2 (4)	6 (22)	3 (13)	
2	1 (1)	1 (2)	0 (0)	0 (0)	
3	0 (0)	0 (0)	4 (15)	5 (21)	

[a]Two patients met criteria for CMR based on v1 lesions despite being graded as i0; 1 patient had chronic active TCMR 1A but did not meet criteria for acute CMR.
[b]C4d assessment was performed by immunofluorescence on frozen sections.
[c]Positive C4d staining was detected in the absence of glomerulitis or peritubular capillaritis in 9 recipients of an ABO-incompatible allograft and was not classified as AMR.
AMR, antibody-mediated rejection; CMR, cell-mediated rejection.

dd-cfDNA provided a better model fit than the more parsimonious model including biopsy scores alone. This observation was supported by both penalized-likelihood criteria (AIC and BIC) favoring the more complex model of biopsy scores with dd-cfDNA. Including the presence of DSA to this model and its interaction with time did not improve fit further (LR test statistic = 3.2; df = 2; $P = 0.20$). Additionally, inclusion of acute inflammation (i, t, and v), microvascular inflammation (g and ptc), and i-IFTA scores to Banff chronicity scores (cg and ci + ct) did not improve model fit (data not shown).

Sensitivity Analysis

There were 9 recipients of an ABO-incompatible transplant with C4d ≥2 who did not have concomitant glomerulitis, peritubular capillaritis, or DSA and were classified as not having AMR. Additionally, there were 5 recipients who had features

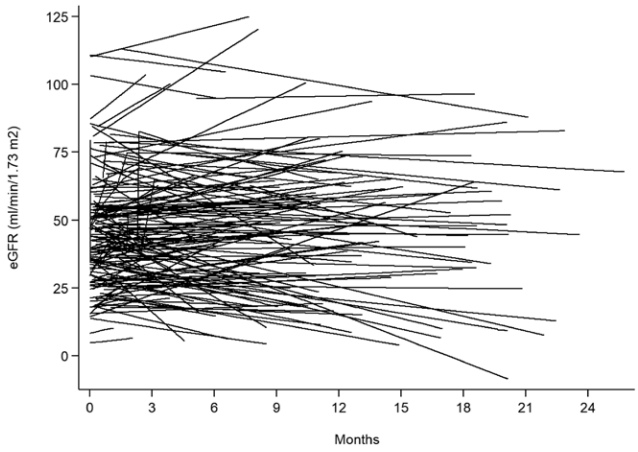

FIGURE 2. Individual eGFR trajectories over time (n = 180). eGFR, estimated glomerular filtration rate.

of BK nephropathy on biopsy, of whom 3 were recipients of an ABO-incompatible transplant. A sensitivity analysis was performed to see if exclusion of these patients resulted in any appreciable difference from what was observed in the overall population (n = 169). The univariate and multivariable regression output is shown in Table S1 SDC, http://links.lww.com/TXD/A261 and comparisons of nested models shown in Table S2, SDC, http://links.lww.com/TXD/A261. The analyses excluding ABO-incompatible recipients and recipients with BK nephropathy observed similar findings to the main analysis.

DISCUSSION

In this study, we found that both Banff biopsy characteristics of chronic transplant glomerulopathy and chronic tubulointerstitial fibrosis were predictive of eGFR over time, and the combination of dd-cfDNA with biopsy characteristics improved model prediction of eGFR over time compared with biopsy characteristics alone. These observations suggest a role for the use of biomarkers, such as dd-cfDNA, in conjunction with kidney transplant biopsies to better inform on prognosis in kidney transplant recipients.

The Banff diagnostic classification has historically relied on clinicopathologic correlations, validating key lesions as characteristic of rejection if associated with disease progression. The concept of C4d-negative AMR was introduced on the basis of studies demonstrating that expression of endothelial-associated and NK cell transcripts was associated with AMR lesions[10,11] and, at least for endothelial-associated transcripts, was a better predictor of graft loss than C4d.[10] The classification of C4d-negative AMR was ultimately validated and incorporated into the Banff 2013 diagnostic classification from data observing that patients with microvascular inflammation found on protocol biopsy in the absence of C4d were more likely to progress to more advanced interstitial fibrosis and tubular atrophy and lower GFR than patients without microvascular inflammation or C4d.[4,12] More recently, the Banff working group has recommended revising the diagnostic classification for borderline CMR to establish a minimum threshold of interstitial inflammation involving 10%–25% of unscarred cortical parenchyma (i1 lesion) in the presence of tubulitis (at minimum t1 lesion).[8] This recommendation was based on 2 reports showing that isolated tubulitis without interstitial inflammation was associated with similar graft survival compared with biopsies without rejection.[13,14]

Additionally, the Banff working group formally recognized molecular markers in the form of validated transcripts or gene classifiers to substitute for DSA for the diagnosis of AMR in the 2017 diagnostic classification.[1] This recognition was an acknowledgment that molecular diagnostics, if thoroughly validated, could be used in conjunction with histology to classify rejection. Recent data also provide support for consideration of clinical characteristics together with histology to more precisely predict outcomes. An unsupervised archetype analysis from Aubert et al[2] demonstrated that integration of functional, immunologic, and histologic characteristics could characterize distinct graft survival outcomes that could not be distinguished by histology alone. Furthermore, a risk prediction score derived from a multivariable Cox regression model incorporating demographic characteristics, DSA specificities and concentrations, and histologic scores validated in an international cohort showed good concordance with observed graft survival (C-statistic, 0.80–0.81).[15]

Our study provides additional support for the paradigm of a multimodal assessment of graft prognosis by demonstrating that assessment of dd-cfDNA together with histologic lesions in a predictive model can better predict eGFR over time compared with histology alone. This suggests that histologic findings might be better interpreted in the context of dd-cfDNA, and consideration of the 2 together may be more informative for making treatment decisions. This concept is supported by recent data indicating that dd-cfDNA can be used to risk stratify patients treated for CMR 1A and borderline CMR lesions on biopsy.[7] In that study, patients with a dd-cfDNA <0.5% exhibited graft stability over 3–6 mo, whereas those with a dd-cfDNA ≥0.5% were more likely to develop DSA and exhibit deterioration in eGFR. Although one cannot conclude from these data that patients with low levels of dd-cfDNA need not be treated for rejection, emerging data are providing support that biomarkers, including dd-cfDNA, should be considered when interpreting biopsies for rejection.

We acknowledge that findings from our study should be validated in larger, multicenter datasets. Baseline characteristics, immunologic risk, and treatment protocols may differ between transplant centers, which may potentially have an impact on eGFR response. Additionally, this was a retrospective study that relied on clinical decisions and not prespecified protocols on dd-cfDNA and biopsy assessment. As such, it is not known whether exclusion of dd-cfDNA tests assessed without a biopsy may have biased our findings. It should also be noted that this study used cross-sectional data and does not account for changes in dd-cfDNA or biopsy findings over time. Despite these limitations, our study lends strength to the paradigm that the diagnostic classification of rejection may be enhanced when biopsies are considered alongside biomarkers, such as dd-cfDNA.

In summary, the combination of Banff cg and ci + ct scores together with dd-cfDNA provided a better model fit for eGFR over time compared with Banff scores alone. Although these findings are preliminary, our study argues that a larger-scale external validation study should be conducted. If validated, assessment of biopsies alongside dd-cfDNA could be

TABLE 3.

(a) Univariate and (b) multivariate associations of Banff biopsy scores and (c) Banff biopsy scores and donor-derived cell-free DNA on eGFR overtime

(a) Predictor	Coefficient (mL/min/1.73 m²/mo; 95% CI)	*P*
Recipient characteristics		
Age at dd-cfDNA assessment (continuous)		
Coefficient: Δ eGFR/mo	−0.8 (−1.8–0.1)[a]	0.09
Age × mo interaction	0.01 (−0.004–0.03)[b]	0.12
Gender (male vs female)		
Reference (female): Δ eGFR/mo	−0.3 (−0.7–0.1)[a]	0.18
Gender × mo interaction	0.3 (−0.2–0.9)[b]	0.28
Donor type (deceased vs living donor)		
Reference (living donor); Δ eGFR/mo	−0.2 (−0.7–0.3)[a]	0.40
Donor type × mo interaction	0.2 (−0.4–0.7)[b]	0.61
Presence of DSA (yes vs no)		
Reference (no DSA): Δ eGFR/mo	−0.004 (−0.3–0.3)[a]	0.98
DSA × mo interaction	−0.3 (−0.8–0.3)[b]	0.33
Banff scores		
cg = 3 vs cg < 3		
Reference (cg < 3): Δ eGFR/mo	−0.02 (−0.3–0.3)[a]	0.87
cg × mo interaction	−1.3 (−2.4 to −0.2)[b]	0.02
ci + ct ≥ 3 vs ci + ct < 3		
Reference (ci + ct < 3): Δ eGFR/mo	0.1 (−0.2–0.4)[a]	0.65
ci + ct × mo interaction	−0.7 (−1.3 to −0.1)[b]	0.03
g + ptc ≥ 2 vs g + ptc < 2		
Reference (g + ptc < 2) Δ eGFR/mo	0.1 (−0.3–0.4)[a]	0.74
g + ptc × mo interaction	−0.4 (−0.9–0.1)[b]	0.14
i + t ≥ 2 vs i + t < 2		
Reference (i + t < 2): Δ eGFR/mo	0.1 (−0.2–0.5)[a]	0.49
i + t × mo interaction	−0.5 (−1.1–0.01)[b]	0.06
i-IFTA = 3 vs i-IFTA < 3		
Reference (i-IFTA < 3): Δ eGFR/mo	0.1 (−0.3–0.4)[a]	0.69
i-IFTA × mo interaction	−0.5 (−1.1–0.04)[b]	0.07
v ≥ 1 vs v < 1		
Reference (v < 1): Δ eGFR/mo	−0.1 (−0.4–0.2)[a]	0.56
v × mo interaction	−0.3 (−1.3–0.7)[b]	0.58
Donor-derived cell-free DNA percentage		
dd-cfDNA (quartiles 3–4 vs 1–2)[c]		
Reference (quartiles 1–2): Δ eGFR/mo	0.1 (−0.2–0.5)[a]	0.47
dd-cfDNA × mo interaction	−0.5 (−1.0–0.1)[b]	0.08
(b)		
Predictor	Coefficient (95% CI)	*P*
Δ eGFR/mo (mL/min/1.73 m²/mo)	0.1 (−0.2–0.4)	0.39
Banff scores		
cg = 3 vs cg < 3 (mL/min/1.73 m²)	−8.7 (−21.5–4.2)	0.19
cg × mo interaction (mL/min/1.73 m²/mo)	−1.2 (−2.3 to −0.2)	0.02
ci + ct ≥ 3 vs ci + ct < 3 (mL/min/1.73 m²)	−9.6 (−16.5 to −2.7)	0.006
ci + ct × mo interaction (mL/min/1.73 m²/mo)	−0.6 (−1.2 to −0.03)	0.04
(c)		
Predictor	Coefficient (95% CI)	*P*
Δ eGFR/mo (mL/min/1.73 m²/mo)	0.4 (−0.04–0.8)	0.08
Banff scores		
cg = 3 vs cg < 3 (mL/min/1.73 m²)	−14.7 (−27.1 to −2.2)	0.02
cg × mo interaction (mL/min/1.73 m²/mo)	−1.1 (−2.2 to −0.0003)	0.05
ci + ct ≥ 3 vs ci + ct < 3 (mL/min/1.73 m²)	−7.1 (−13.7 to −0.4)	0.04
ci + ct × mo interaction (mL/min/1.73 m²/mo)	−0.7 (−1.3 to −0.1)	0.02
Donor-derived cell-free DNA percentage		
Quartiles 3–4 vs 1–2 (mL/min/1.73 m²)[c]	13.4 (7.7–19.1)	<0.001
dd-cfDNA (quartiles 3–4 vs 1–2) × mo interaction (mL/min/1.73 m²/mo)	−0.4 (−1.0–0.1)	0.10

[a]Reference coefficient (Δ eGFR/mo).
[b]Interaction terms are interpreted as the difference in eGFR slope between the reference and comparator groups. Δ eGFR/mo for the comparator group equals the sum of the coefficients for the reference group and the interaction.
[c]dd-cfDNA range: quartiles 1–2: 0.15%–0.68%; quartiles 3–4: 0.71%–16%.
CI, confidence interval; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; i-IFTA, inflammation in area of interstitial fibrosis and tubular atrophy.

TABLE 4.

Comparison of model fit between nested models. A lower AIC and BIC indicate better model fit

	Likelihood-ratio test				
	Likelihood-ratio test statistic	df	*P*	AIC	BIC
Model 1[a]	21.1	2	<0.001	18684	18742
Model 2[b]				18666	18736

[a]Model 1: $eGFR = \alpha + \beta_1(cg^{\dagger}) + \beta_2(mo) + \beta_3(cg^{\dagger} \times mo) + \beta_4(ci + ct^{\ddagger}) + \beta_5[(ci + ct^{\ddagger}) \times (mo)]$ (Table 3).
[b]Model 2: $eGFR = \alpha + \beta_1(cg^{\dagger}) + \beta_2(mo) + \beta_3(cg^{\dagger} \times mo) + \beta_4(ci + ct^{\ddagger}) + \beta_5[(ci + ct^{\ddagger}) \times (mo)] + \beta_6(dd\text{-}cfDNA) + \beta_7(dd\text{-}cfDNA \times mo)$ (Table 3).
$^{\dagger}cg = 3$ vs $cg < 3$
$^{\ddagger}ci + ct \geq 3$ vs $ci + ct < 3$
AIC, Akaike Information Criterion; BIC; Bayesian Information Criterion; dd-cfDNA, donor-derived cell-free DNA; eGFR, estimated glomerular filtration rate.

considered as a newer paradigm for classifying rejection in kidney transplantation.

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