Cross-Sectional Study

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Donor-derived cell-free DNA for reducing unnecessary biopsies in stable dnDSA-positive kidney transplant recipients: a multicenter, prospective study

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Background: In clinically stable kidney transplant recipients with de novo donor-specific anti-HLA antibodies (dnDSA), only 30 ~ 40% exhibit biopsy-proven rejection, leading to a substantial number of unnecessary biopsies. Donor-derived cell-free DNA (dd-cfDNA) has emerged as a promising noninvasive biomarker of allograft injury. Nevertheless, its clinical utility for guiding biopsy decisions in dnDSA-positive stable kidney transplant recipients remains uncertain. This prospective multicenter cross-sectional study evaluated whether dd-cfDNA offers incremental predictive value for subclinical rejection in dnDSA-positive recipients with stable graft function.

Methods: A total of 123 adult kidney transplant recipients with stable renal function were enrolled, comprising 46 dnDSA-negative and 77 dnDSA-positive patients. All participants underwent both dd-cfDNA and dnDSA testing, along with protocol or indication biopsies. Recipients with ABO-incompatible or preformed DSA-positive transplants were excluded. The diagnostic performance of dnDSA alone versus dnDSA combined with dd-cfDNA for predicting biopsy-proven subclinical rejection was assessed using receiver operating characteristic curve analysis.

Results: Median dd-cfDNA levels were higher in dnDSA-positive patients (1.2% [IQR: 0.4–1.8]) compared with dnDSA-negative patients (0.3% [IQR: 0.2–0.4]). Subclinical rejection was histologically confirmed in 37 patients. Among dnDSA-positive recipients, combining dd-cfDNA ≥ 1.0 with dnDSA markedly improved diagnostic accuracy (AUC: 0.81; 95% CI: 0.74–0.88) compared with dnDSA alone (AUC: 0.74; 95% CI: 0.68–0.82). This combined approach achieved a positive predictive value of 46.2% and a negative predictive value of 97.8%. Moreover, elevated Banff microvascular inflammation (MVI) scores showed a strong correlation with higher dd-cfDNA levels ($P < 0.001$).

Conclusions: In dnDSA-positive kidney transplant recipients with stable renal function, dd-cfDNA provides incremental diagnostic value for identifying subclinical rejection and reflects the degree of microvascular injury. The integration of dd-cfDNA with dnDSA may enable more targeted and judicious use of biopsies, thereby reducing procedural burden while maintaining diagnostic precision.

Keywords: de novo DSA (dnDSA), donor-derived cell-free DNA (dd-cfDNA), kidney transplantation, subclinical rejection

Introduction

Subclinical rejection remains a major determinant of long-term graft failure in kidney transplant recipients[1–4]. Microvascular inflammation (MVI) has been shown to more than double the risk of allograft loss and is recognized as a key pathological driver of diminished graft longevity[5–8]. Accordingly, early detection and prompt management of subclinical rejection and MVI are essential for improving long-term outcomes.

Conventional diagnostic strategies, however, remain limited. Although kidney biopsy is considered the diagnostic gold standard, it is invasive and carries procedural risks[9]. Serum creatinine levels typically rise only after substantial allograft injury, while proteinuria lacks specificity. Furthermore, donor-specific antibody (DSA) testing alone remains insufficient for reliable clinical decision-making: only 30–40% of DSA-positive patients exhibit biopsy-proven rejection, resulting in a high rate of unnecessary biopsies and delayed diagnosis[10,11].

Donor-derived cell-free DNA (dd-cfDNA) has recently emerged as a promising noninvasive biomarker capable of overcoming these limitations[12]. Previous studies have demonstrated that dd-cfDNA can detect allograft injury and rejection months earlier than traditional markers, with high sensitivity and specificity[13,14]. Moreover, dd-cfDNA levels have been associated with long-term outcomes and severe MVI[15–18].

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Nevertheless, the potential of dd-cfDNA to reduce unnecessary biopsies in dnDSA-positive kidney transplant recipients with stable renal function has not been comprehensively evaluated. In addition, most prior studies have focused on symptomatic patients, leaving the role of dd-cfDNA in clinically stable population largely undefined. This multicenter study was designed to address these gaps by assessing the combined diagnostic performance of dd-cfDNA and dnDSA in predicting histologic microvascular injury and subclinical rejection among stable kidney transplant recipients. Our goal is to provide evidence supporting dd-cfDNA as a noninvasive risk-stratification tool to guide biopsy decision-making in clinical practice.

HIGHTLIGHTS

- In de novo donor-specific antibodies (dnDSA)-positive stable kidney transplant recipients, only 30–40% have biopsy-proven rejection, leading to many unnecessary biopsies.
- 123 adult kidney transplant recipients (77 dnDSA-positive, 46 dnDSA-negative) with stable renal function were prospectively enrolled across three Korean centers, all undergoing donor-derived cell-free DNA (dd-cfDNA) testing and allograft biopsy.
- Adding dd-cfDNA ≥1.0% to dnDSA improved AUC from 0.74 to 0.81, with a negative predictive value of 97.8%, enabling safe deferral of biopsy in most low-risk dnDSA-positive patients.
- dd-cfDNA strongly correlated with Banff microvascular inflammation scores ($P < 0.001$), reflecting the degree of microvascular injury in the allograft.
- Integrating dd-cfDNA with dnDSA offers a noninvasive, risk-stratified strategy to reduce unnecessary biopsies and optimize post-transplant surveillance.

Method

Study design and population

This multicenter, prospective cross-sectional study enrolled kidney transplant recipients from three institutions in Korea who underwent transplantation between January 2010 and June 2020 (https://clinicaltrials.gov/ct2/show/NCT06013358). Eligible participants were adults aged 18 years or older with stable renal function following transplantation. Stable renal function was defined as a change in serum creatinine of less than 15% compared with measurements obtained 6 months earlier. Both dnDSA-positive and dnDSA-negative patients were included. dnDSA positivity was defined as the presence of donor-specific anti-HLA class I or II antibodies with a mean fluorescence intensity (MFI) ≥ 1000. Exclusion criteria included recipients of multi-organ transplantation, ABO-incompatible transplants, preformed DSA positivity at the time of the transplantation, and pediatric recipients younger than 18 years at the time of transplantation (Fig. 1).

Sample and data collection

Monitoring of dnDSA is performed at 1, 2, and 4 weeks, then every 2 months up to 1 year post-transplantation, followed by every 6 months until 3 years, and annually thereafter. Patients with dnDSAs exhibiting an MFI ≥ 1000 underwent indication biopsies, during which both dd-cfDNA and dnDSA levels were measured. Likewise, patients undergoing protocol biopsies at 1 and 5 years post-transplantation were also evaluated for dnDSAs and dd-cfDNA levels. Protocol biopsies were performed according to institutional policies. Clinical data including demographic variables, donor and recipient characteristics, serum creatinine, estimated glomerular filtration rate, urinary protein analysis, DSA status, and infection history were collected for all participants.

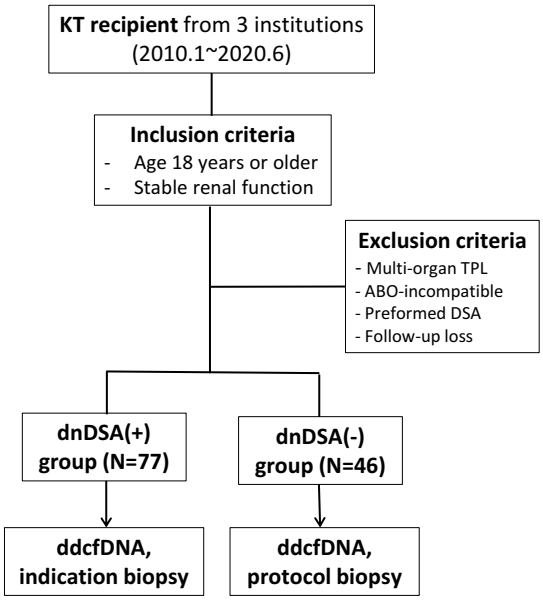

Figure 1. Flow chart of the study.

dd-cfDNA assay

The dd-cfDNA assay was performed using the AlloSeq cfDNA assay (CareDx, Brisbane, CA, USA). Peripheral blood samples were collected before biopsy in Streck Cell-Free DNA BCT tubes. Using the AlloSeq cfDNA platform, DNA single-nucleotide polymorphisms were amplified, followed by size selection and purification of the amplification products. The final products underwent next-generation sequencing for analysis. The relative percentage of dd-cfDNA was calculated as a fraction of total cfDNA[18].

Study endpoints

The primary endpoints were the diagnostic performance of dd-cfDNA and dnDSA levels for detecting subclinical rejection and histologic MVI. Biopsy-proven acute rejection (BPAR) was defined according to the Banff 2019 classification[19]. For this study, "any rejection" referred to all forms of rejection, excluding borderline changes.

Statistical analysis

Descriptive statistics were reported as means ± standard deviations or medians with interquartile ranges, as appropriate.

Group comparisons were performed using Student's t-test, Mann–Whitney U test, or Chi-square test. The diagnostic performance of dd-cfDNA and dnDSA was assessed using receiver operating characteristic (ROC) curve analysis, with calculation of area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Statistical significance was set at $P < 0.05$. Analyses were performed using R software and RStudio (R version 4.5.1).

Ethical considerations

This study was approved by the Institutional Review Boards of all participating institutions. The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment. The study has been reported in line with the STROCSS 2025 criteria[20].

Result

Baseline characteristics of enrolled patients

A total of 123 kidney transplant recipients were enrolled across the three participating centers, including 77 dnDSA-positive and 46 dnDSA-negative patients. The median age was lower in the dnDSA-positive group than in the dnDSA-negative group (47 vs 51 years, $P = 0.014$). Regarding donor-recipient relationship, there were no significant differences between the dnDSA(+) and dnDSA(−) groups ($P = 0.298$). Additionally, the prevalence of comorbidities, including diabetes mellitus ($P = 0.235$) and hypertension ($P = 0.788$), did not differ significantly between the cohorts (Table 1). Median dd-cfDNA levels were significantly higher in the dnDSA-positive patients than in the dnDSA-negative patients (1.2% [IQR: 0.4–1.8] vs 0.3% [IQR: 0.2–0.4]; $P < 0.001$). The median duration of follow-up was significantly longer in the dnDSA(+) group and compared to the dnDSA(−) group (85.0 [41.5–127.5] months vs 12.9 [11.0–14.0] months, $P<0.001$)

Distribution of dd-cfDNA according to allograft pathology

Among the study population, 77 patients with dnDSA positivity underwent indication biopsies, whereas 46 dnDSA-negative patients underwent protocol biopsies. Blood samples for dd-cfDNA measurement were obtained within 24 hours before biopsy in all cases. Overall, histologically confirmed subclinical rejection was identified in 37 patients (30.1%).

The histopathologic diagnosis of allograft kidney was compared between dnDSA-positive and dnDSA-negative group. Thirty-six patients suffered subclinical rejection in dnDSA-positive group and only one patient underwent subclinical rejection in dnDSA-negative group (46.8% vs 2.2%, $P <0.001$) (Table 2). The distribution of dd-cfDNA levels varied significantly among histopathologic diagnosis (Fig. 2). In the dnDSA-positive cohort, 34 patients (44.2%) were diagnosed with antibody-mediated rejection (ABMR), with a median dd-cfDNA level of 1.5%. The number of patients with no rejection in dnDSA-positive group was 28 (36.4%), and the median dd-cfDNA level of them was 0.54%. There were no statistically significant differences in dd-cfDNA levels between histopathologic diagnosis in both dnDSA-positive and negative groups. Patients with mixed rejection demonstrated a higher median dd-cfDNA level of 2.4%. Among 13 patients categorized as "other diagnoses," IgA nephropathy was the most common finding (8 patients, 10.4%), with a median dd-cfDNA of 0.79%. In contrast, within the dnDSA-negative cohort, ABMR was rare, occurring only in one patient (2.2%) with a dd-cfDNA level of 0.11%. A small number of patients with IgA nephropathy and glomerulonephropathy showed a median dd-cfDNA of 0.88%.

ROC curve analysis for rejection

ROC curves were generated to evaluate the ability of each marker to predict any rejection in patients with stable renal function (Table 3). The AUC for dnDSA alone was 0.74 (95% CI: 0.68–0.80) with a PPV of 46.2% and an NPV of 97.8%. Adding different cut-off levels of dd-cfDNA to the dnDSA positivity improved AUC above 0.8. When dd-cfDNA ≥ 0.7% was combined with positive dnDSA, the AUC improved to 0.82 (95% CI: 0.75–0.89) with a PPV of 59.2% and an NPV of 89.2%. When dd-cfDNA ≥ 1.0% was combined with positive dnDSA, the AUC was 0.81 (95% CI: 0.74–0.88), with a PPV of 46.2% and 97.8%. The statistical difference between AUCs was evaluated by DeLong's test in combination with dd-cfDNA level with dnDSA positivity ($P <0.05$). In contrast, serum creatinine, urine protein-to-creatinine ratio, and dnDSA MFI each demonstrated AUCs below 0.70, indicating limited discriminatory performance. These findings are summarized in Figure 3 and Table 3.

Association of dd-cfDNA with Banff criteria

Correlation analyses between dd-cfDNA and individual Banff lesion scores revealed selective associations with specific histopathologic features (Fig. 4a–p). Banff scores for tubulities (t), intimal arteritis (v), C4d staining, chronic tubular atrophy (ct), chronic vascular changes (cv), transplant glomerulopathy (cg), arteriolar hyalinosis (ah), acute arteriolar hyalinosis (aah), total inflammation (ti), and interstitial fibrosis and tubular atrophy (iFTA) showed no statistically significant correlation with dd-cfDNA levels. However, dd-cfDNA levels were strongly correlated with Banff glomerulitis (g) and peritubular capillaritis (ptc) scores ($P < 0.05$). Additionally, Banff chronic inflammation (ci) and mesangial matrix expansion (mm) scores demonstrated partial associations, with dd-cfDNA elevation in specific severity ranges. MVI score showed a robust positive correlation with dd-cfDNA levels ($P <0.001$). Median dd-cfDNA levels were 0.54% (IQR: 0.26–1.29) for MVI scores 0–1, 1.67% (IQR: 1.36–2.27) for MVI scores 2–3, and 1.60% (IQR: 1.27–2.45) for MVI scores ≥ 4.

Discussion

Our study demonstrated the incremental diagnostic value of dd-cfDNA for detecting any rejection in dnDSA-positive kidney transplant recipients with stable renal function. When used alone, dnDSA identified rejection with an AUC of 0.74, whereas combining dnDSA with dd-cfDNA ≥ 0.7% significantly

Table 1

Baseline demographics.

	dnDSA+ (*n* = 77)	dnDSA− (*n* = 46)	*P*-value
Male, *n*(%)	55 (71.4)	28 (60.9)	0.226
Age at transplant, year, median (IQR)	47.0 (35.5–54.0)	51.0 (42.5–60.3)	0.014
BMI (kg/m^2)	22.8 (20.8–25.5)	22.7(19.9–25.5)	0.676
DM	12 (15.6)	12 (26.1)	0.235
HTN	59 (76.6)	37 (80.4)	0.788
Primary causes of ESRD, *n*(%)			0.129
DM	8 (10.4)	11 (23.9)	
HTN	11 (14.3)	3 (6.5)	
PKD	3 (3.9)	2 (4.3)	
IgAN	17 (22.1)	12 (26.1)	
FSGS	7 (9.1)	4 (8.7)	
Other GN	1 (1.3)	3 (6.5)	
Unknown	20 (26.0)	8 (17.4)	
Others	10 (13.0)	3 (6.5)	
Pretransplant PRA >50%	1 (1.3)	0 (0.0)	1.000
Induction agent			0.016
Basiliximab	67 (87.0)	46 (100.0)	
ATG	8 (10.4)	0 (0.0)	
Basiliximab & ATG	2 (2.6)	0 (0.0)	
CNI agent			0.409
Tacrolimus	72 (93.5)	45 (97.8)	
mTOR inhibitor	5 (6.5)	1 (2.2)	
Retransplant, *n*(%)	4 (5.2)	2 (4.3)	1.000
Donor relation, *n*(%)			0.298
Living donor	52 (67.5)	34 (73.9)	
First-degree relative	17 (22.1)	11 (23.9)	
Second-degree relative	9 (11.7)	12 (26.1)	
Fourth-degree relative	3 (3.9)	1 (2.2)	
Unrelated (including spouse)	23 (29.9)	10 (21.7)	
Deceased donor	25 (32.5)	12 (26.1)	
Desensitization	4 (5.2)	0 (0.0)	0.296
dnDSA, *n*(%)			<0.001
HLA class I	9 (11.7)		
HLA class II	60 (77.9)		
HLA class I & II	8 (10.4)		
dnDSA MFI, *n*(%)			<0.001
Weak (1000–3000)	33 (42.9)	0 (0.0)	
Intermediate (3000–10 000)	23 (29.9)	0 (0.0)	
Strong (>10 000)	21 (27.3)	0 (0.0)	
dd-cfDNA(%), median (IQR)	1.2 (0.4–1.8)	0.3 (0.2–0.4)	<0.001
Duration of follow up (month), median (IQR)	85.0 (41.5–127.5)	12.9 (11.0–14.0)	<0.001

ATG, anti-thymocyte globulin; BMI, body mass index; CNI, calcineurin inhibitor; dnDSA, de novo donor-specific antibody; DM, diabetes mellitus; ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; HTN, hypertension; IgAN, immunoglobulin A nephropathy; IQR, interquartile range; MFI, mean fluorescence intensity; PRA, panel reactive antibody; PKD, polycystic kidney disease; SD, standard deviation.
Categorical variables were described in *n*(%).
Continuous variables ware described in median (IQR).

Table 2

Histopathologic diagnosis of allograft kidney.

Pathologic diagnosis	dnDSA+ (*n* = 77)	dnDSA− (*n* = 46)	*P*-value
No rejection, *n*(%)	28 (36.4)	42 (91.3)	<0.001
ABMR, *n*(%)	34 (44.2)	1 (2.2)	
Mixed (ABMR&TCMR), *n*(%)	2 (2.6)	0 (0.0)	
Others			
IgAN, *n*(%)	8 (10.4)	2 (4.3)	
GN recur, *n*(%)	1 (1.3)	1 (2.2)	
Amyloidosis, *n*(%)	1 (1.3)	0 (0.0)	
DM nephropathy, *n*(%)	2 (2.6)	0 (0.0)	
CNI toxicity, *n*(%)	1 (1.3)	0 (0.0)	

ABMR, antibody-mediated rejection; CNI, calcineurin inhibitor; DM, diabetes mellitus; dnDSA, de novo donor-specific antibody; GN, glomerulonephritis; IgAN, immunoglobulin A nephropathy; TCMR, T cell-mediated rejection.
Categorical variables were described in *n*(%).

improved the AUC to 0.82 ($P < 0.001$). Notably, when combining dd-cfDNA ≥ 1.0% in dnDSA-positive patients, the AUC for detecting subclinical rejection further increased from 0.74 to 0.81 compared to dnDSA alone, while both NPV and PPV remained comparable. The statistical difference between AUCs was evaluated by DeLong's test ($P < 0.05$). The improvement indicates that dd-cfDNA complements dnDSA by enhancing diagnostic discrimination without sacrificing predictive performance. Therefore, utilizing dd-cfDNA ≥ 1.0% in dnDSA-positive patients withstable renal function provide evidence for the role in refining stratification and biopsy decision in the clinical challenging population.

Previous studies have also highlighted the utility of combining DSA and dd-cfDNA for diagnosing rejection. Jordan SC *et al* first reported that while DSA alone yielded a PPV of 48%, the addition of dd-cfDNA (at 1%) increased the PPV to 89% and the NPV to 83%[21]. Similarly, Olivier Aubert *et al* demonstrated that integrating dd-cfDNA into standard monitoring parameters, including dnDSA, improved discrimination even in clinically stable recipients, achieving an AUC of 0.84[22]. These studies align with our findings and reinforce the clinical relevance of combining dd-cfDNA with dnDSA as a robust, non-invasive strategy to optimize rejection monitoring in kidney transplantation.

In our study, subclinical rejection was observed in 46.8% of dnDSA-positive recipients with stable renal function. Reported rejection rates in such populations have varied widely across studies. For example, a French multicenter study by Bertrand D *et al* found subclinical AMR in 49% of 123 dnDSA-positive recipients[23], whereas Waldecker *et al* reported a higher rejection rate of 91.7% among 84 dnDSA-positive recipients in a German single-center cohort, noting that 29 of 34 clinically stable patients (85.3%) had BPAR[24]. Such variability likely reflects differences in patient demographics, immunosuppressive protocols, and dnDSA strength. The European Society for Organ Transplantation consensus statement on DSA monitoring recommends testing every 3–6 months and suggests biopsy consideration in dnDSA-positive patients[25]. Nonetheless, clinicians continue to face a dilemma when deciding whether to perform invasive procedures in otherwise stable recipients. Our findings address this issue by offering a practical solution: dd-cfDNA testing can identify the majority of dnDSA-positive patients (NPV 89.2%) who may safely defer biopsy, thereby reducing unnecessary procedures.

We also found a strong correlation between dd-cfDNA levels and Banff MVI scores ($P < 0.001$), suggesting dd-cfDNA may serve as a quantitative indicator of microvascular injury. This

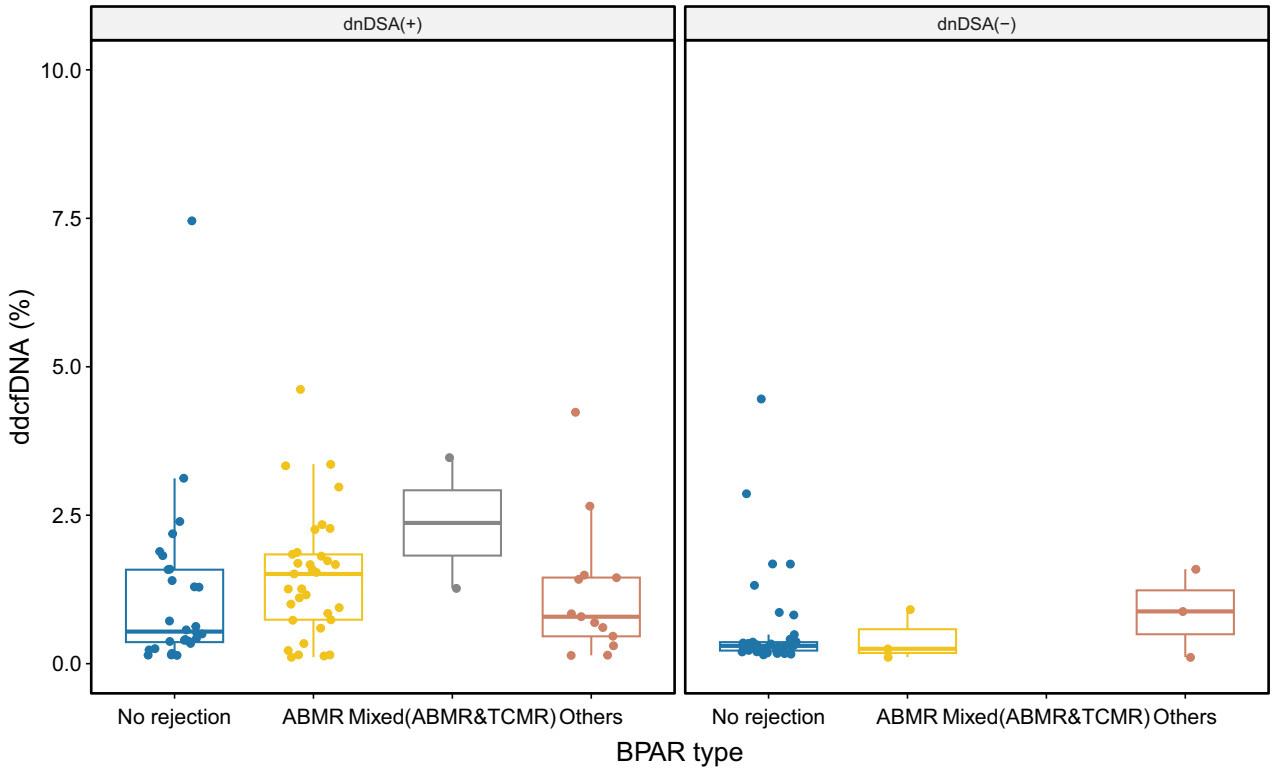

Figure 2. dd-cfDNA levels according to pathologic diagnosis. (A) dnDSA (+) group. (B) dnDSA (–) group.

association supports the use of dd-cfDNA as an objective biomarker where conventional histologic grading may be limited by inter-observer variability. Kim HD *et al* similarly reported a strong relationship between dd-cfDNA and severe MVI (AUC: 0.855)[16]. Furthermore, Akifova *et al* demonstrated in a randomized controlled trial that a dd-cfDNA–guided early biopsy strategy reduced time to AMR diagnosis by nearly 80%, underscoring the clinical utility[26]. Collectively, these findings extend the role of dd-cfDNA beyond a diagnosis, suggesting its potential as a tool to guide therapeutic intensity according to the degree of MVI. This is particularly relevant in subclinical rejection, where MVI severity directly predicts long-term allograft outcomes.

Our ethnically homogenous Korean cohort provides novel insights into the immunologic and dd-cfDNA response characteristics in Asian kidney transplant recipients. To date, data on dd-cfDNA in kidney transplantation in Asian population remain scarce[16,27]. By evaluating dd-cfDNA in Korean kidney

Table 3

AUC, sensitivity, specificity, PPV, and NPV of variables.

Variable	AUC (95% CI)	*P*-value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
dd-cfDNA	0.70(0.59–0.81)	<0.001	78.4	67.4	50.9	87.9
dnDSA	0.74 (0.68–0.80)	<0.001	97.3	51.2	46.2	97.8
dnDSA_MFI	0.62(0.49–0.75)	<0.001	52.8	75.0	65.5	63.8
dd-cfDNA ≥0.5 + dnDSA(+)	0.79 (0.72–0.86)	<0.001	81.1	69.8	53.6	89.6
dd-cfDNA ≥0.6 + dnDSA(+)	0.81 (0.74–0.88)	<0.001	81.1	73.3	56.6	90.0
dd-cfDNA ≥0.7 + dnDSA(+)	0.82(0.75–0.89)	<0.001	78.4	76.7	59.2	89.2
dd-cfDNA ≥0.8 + dnDSA(+)	0.82(0.75–0.89)	<0.001	73.0	79.1	60.0	87.2
dd-cfDNA ≥0.9 + dnDSA(+)	0.82(0.75–0.89)	0.784	70.3	80.2	60.5	86.2
dd-cfDNA ≥1.0 + dnDSA(+)	0.81(0.74–0.88)	<0.001	97.3	51.2	46.2	97.8
Serum creatinine (mg/dl)	0.65(0.54–0.76)	0.784	64.9	66.3	45.3	81.4
UPCR	0.67(0.54–0.80)	<0.001	48.4	82.9	68.2	68.0

AUC, area under the curve; CI, confidence interval; dd-cfDNA, donor-derived cell-free DNA; NPV, negative predictive value; PPV, positive predictive value; UPCR, urine protein-to-creatinine ratio.

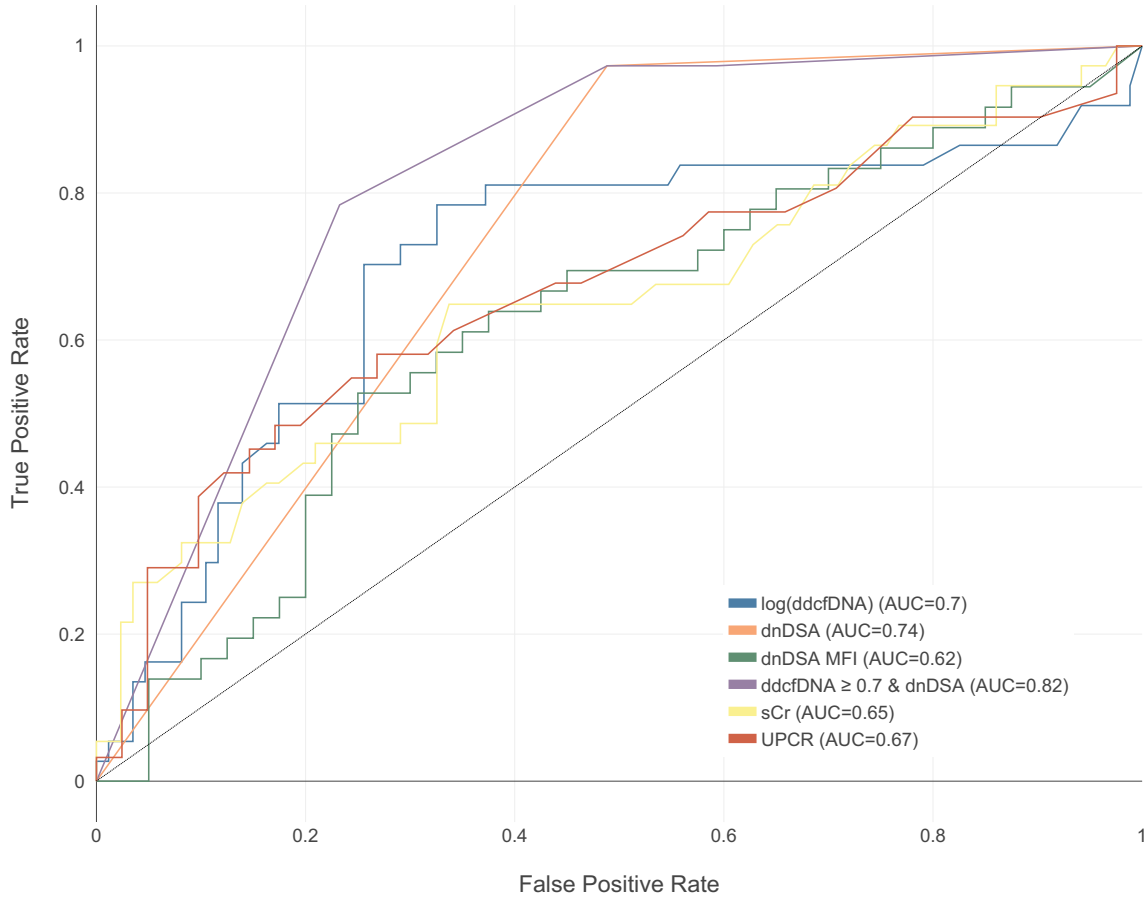

Figure 3. ROC curves for discriminating any rejection.

recipients with stable graft function, our study enriches the limited evidence in Asian population, strengthening the dd-cfDNA application across diverse ethinic and geographic contexts.

This multicenter study is among the first to validate the combined diagnostic utility of dd-cfDNA and dnDSA for detecting subclinical rejection and MVI in a homogenous Asian population. By demonstrating an improvement in AUC, we provide robust evidence supporting a noninvasive, risk-stratified approach that can safely reduce unnecessary biopsies. Integration of dd-cfDNA into post-transplant surveillance protocols could enhance patient safety and yield significant healthcare cost savings by minimizing unwarranted biopsy procedures in the majority of dnDSA-positive stable recipients.

There are several limitations to this study. First, the sample size was relatively small, which may have limited the statistical power to support our findings. Second, dd-cfDNA was measured at a single different time point. Given that previous longitudinal studies have shown dynamic fluctuations in dd-cfDNA levels[15,28], larger prospective studies with serial sampling are warranted. Third, the fractional level of dd-cfDNA (%) may vary over time post-transplantation due to changes in total cfDNA levels[29].

The discrepancy in the duration of follow-up between two groups is a potential confounding factor in our analysis (85.0 vs 12.9 months, P <0.001). Consequently, future studies with larger cohorts are required to perform time-adjusted analyses to validate the diagnostic efficacy of dd-cfDNA relation to the post-transplant interval. Fourth, high-risk transplant recipients, such as ABO-incompatible transplants, were excluded, which may limit generalizability. Lastly, variations in dnDSA screening and biopsy protocols among participating centers may have influenced the detection rates of subclinical rejection.

Conclusion

In conclusion, this multicenter study demonstrates that dd-cfDNA testing can effectively reduce unnecessary biopsies in dnDSA-positive kidney transplant recipients with stable renal function. Incorporating dd-cfDNA into post-transplant monitoring protocols offers a practical, cost-effective, and evidence-

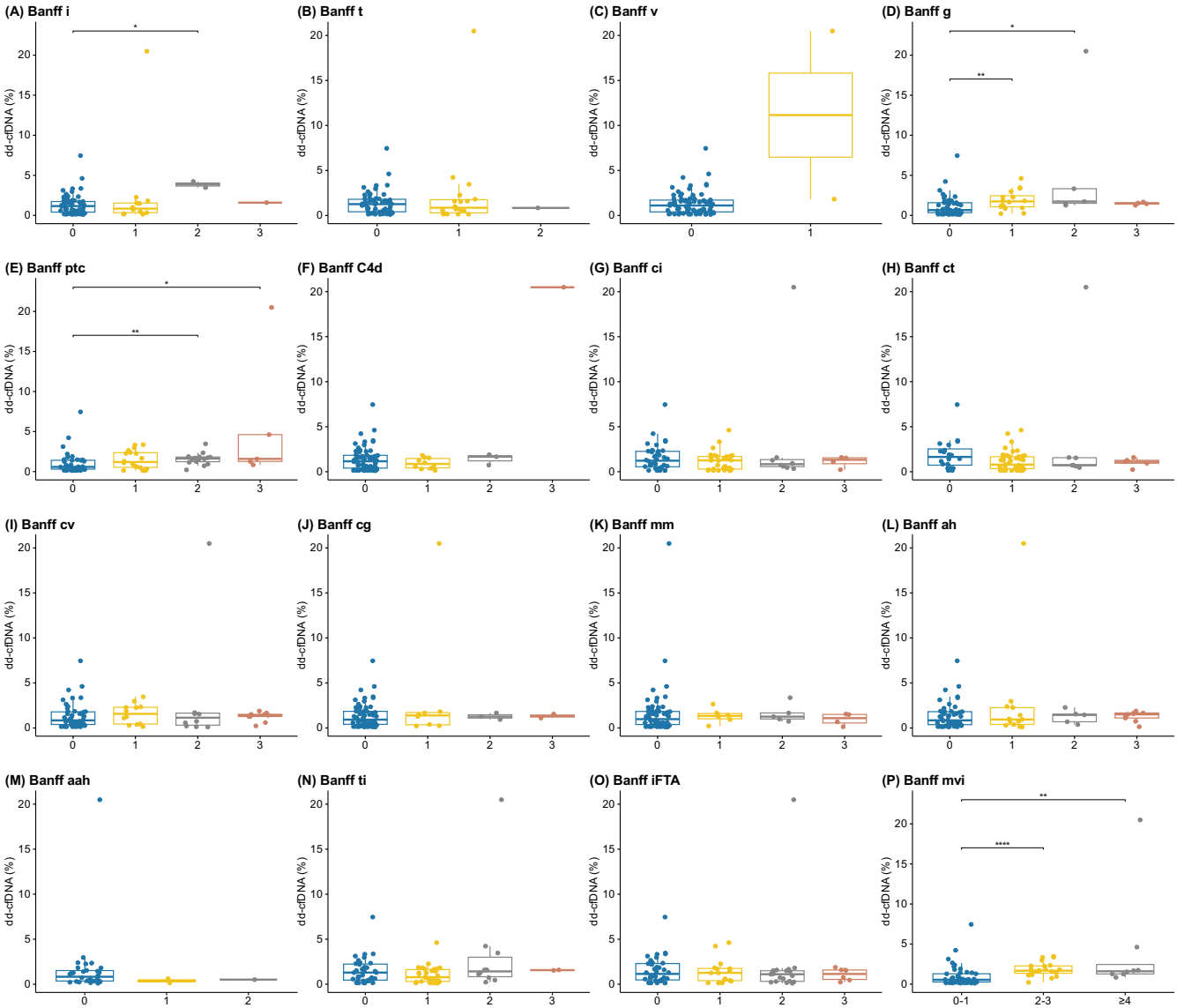

Figure 4. Association of dd-cfDNA with Banff criteria. (A) Banff i, (B) Banff t, (C) Banff v, (D) Banff g, (E) Banff ptc, (F) Banff c4d, (G) Banff ci, (H) Banff (ct), (I) Banff cv, (J) Banff cg, (K) Banff mm, (l) Banff ah, (M) Banff aah, (N) Banff ti, (J) Banff iFTA, (P) Banff mvi. Data are presented as median dd-cfDNA (%) according to the severity grade of each Banff lesion score. Statistical significance was assessed using appropriate non-parametric tests, and significance levels are indicated as ns (not significant, *P*-value > 0.05), * (*P*-value ≤ 0.05), ** (*P*-value ≤ 0.01), and *** (*P*-value ≤ 0.001).

based approach to optimize rejection surveillance and improve the overall quality of post-transplant care in kidney transplantation.

Ethical approval

The study followed the ethical principles of the Helsinki Declaration, and the institutional ethics review board approved this cohort study (SNUH IRB No. 2212-098-1386, Severance IRB No. 4-2023-0076, and KUMC IRB No. 2023AN0123).

Consent

Written informed consent was obtained from all participants before enrollment.

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Author contributions

Conceptualization was conducted by S.M. and A.C., while data curation was performed by A.C., J.L., and C.W.J. Formal analysis was done by A.C. and S.K., and funding acquisition was the responsibility of S.M.. Methodology was developed by A.H., S.M., Y.C.K., and S.P., and the original draft was written by A.C.. Writing review and editing were undertaken by S.M., A.H., and J.H., and visualization was performed by A.C. and S.K.

Conflicts of interest disclosure

The authors declare no conflicts of interest.

Research registration unique identifying number (UIN)

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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