Supplemental Digital Content

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Table S1: Performance of the dd-cfDNA fraction and quantity as determined by both MMDx and histology across total, training, and test sets.

Diagnostic modality	Sample set	Two-Threshold Algorithm					Logistic Regression
		Sensitivity (%; X/X)	Specificity (%; X/X)	PPV (%, X/X)	NPV (%, X/X)	Accuracy (%, X/X)	AUC
MMDx	Total (N=367)	82.4 103/125	79.7 193/242	67.8 103/152	89.8 193/215	80.1 294/367	0.86*
	Training (N=149)	81.5 44/54	77.9 74/95	67.8 44/65	88.1 74/84	79.19 118/149	0.84*
	Test (N=218)	83.1 59/71	81.0 119/147	67.8 59/87	90.8 119/131	81.65 178/218	0.88
Banff Histology	Total (N=359)	73.2 104/142	79.3 172/217	69.8 104/149	81.9 172/210	76.9 312/359	0.82*
	Training (N=146)	72.9 43/59	77.0 67/87	68.3 43/63	80.7 67/83	75.3 110/146	0.82*
	Test (N=213)	73.5 61/83	80.8 105/130	70.9 61/86	82.7 105/127	77.9 166/213	0.82

*AUC calculated by 10 fold cross validation of logistic regression model

Table S2: Positive and negative predictive values for the two-threshold algorithm projected to different cohort AR prevalences, using molecular pathology as a comparator.

Cohort Prevalence (%)	PPV (%)	NPV (%)
10.0	32.6	97.7
15.0	43.5	96.4
20.0	52.2	95.0
25.0	59.2	93.5
32.6*	67.8	90.8

*actual prevalence in study cohort

Table S3: Published prospective studies assessing the performance of dd-cfDNA to detect rejection in renal allograft patients.

Study	dd-cfDNA measure	Biopsymatched samples in the analysis	Samples with biopsyproven AR in analysis	Sensitivity	Specificity	AUC
Bloom 2017	fraction	107	27	59%	85%	0.74
Sigdel 2018	fraction	217	35	89%	73%	0.87
Huang 2019	fraction	63	34	79%	72%	0.71
Oellerich 2019	quantity	143	22	73%	73%	0.83
Gupta 2021*	fraction	208	92	52%	92%	0.80
Current study*	both	367	125	82%	80%	0.86

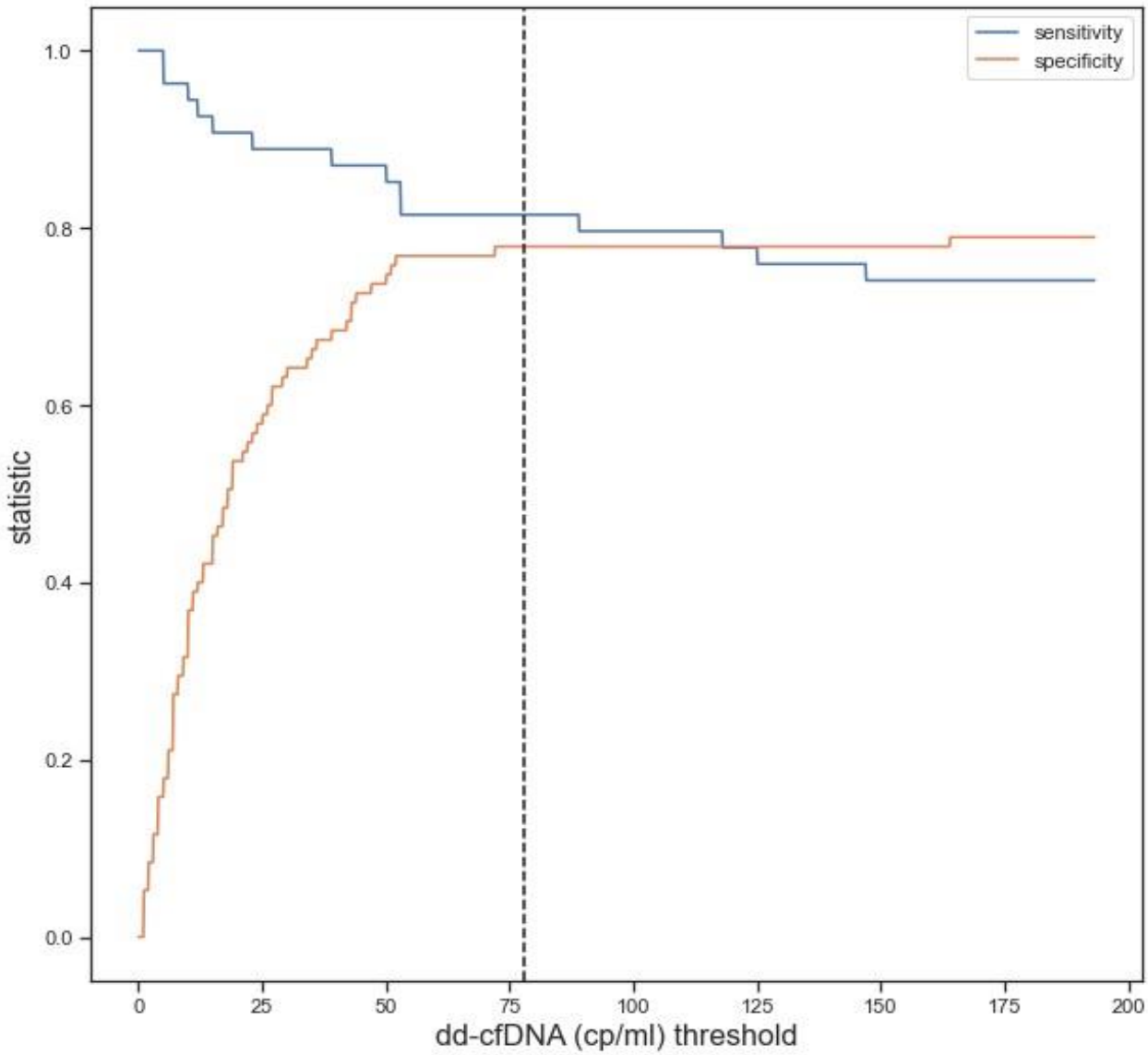

Figure S1: The numerical value for the dd-cfDNA quantity threshold was chosen by examination of the sensitivity (blue line) and specificity (red line) of the training set while keeping the dd-cfDNA fraction threshold constant at 1%. The vertical dashed line shows the final choice of the threshold value, 78 cp/mL.

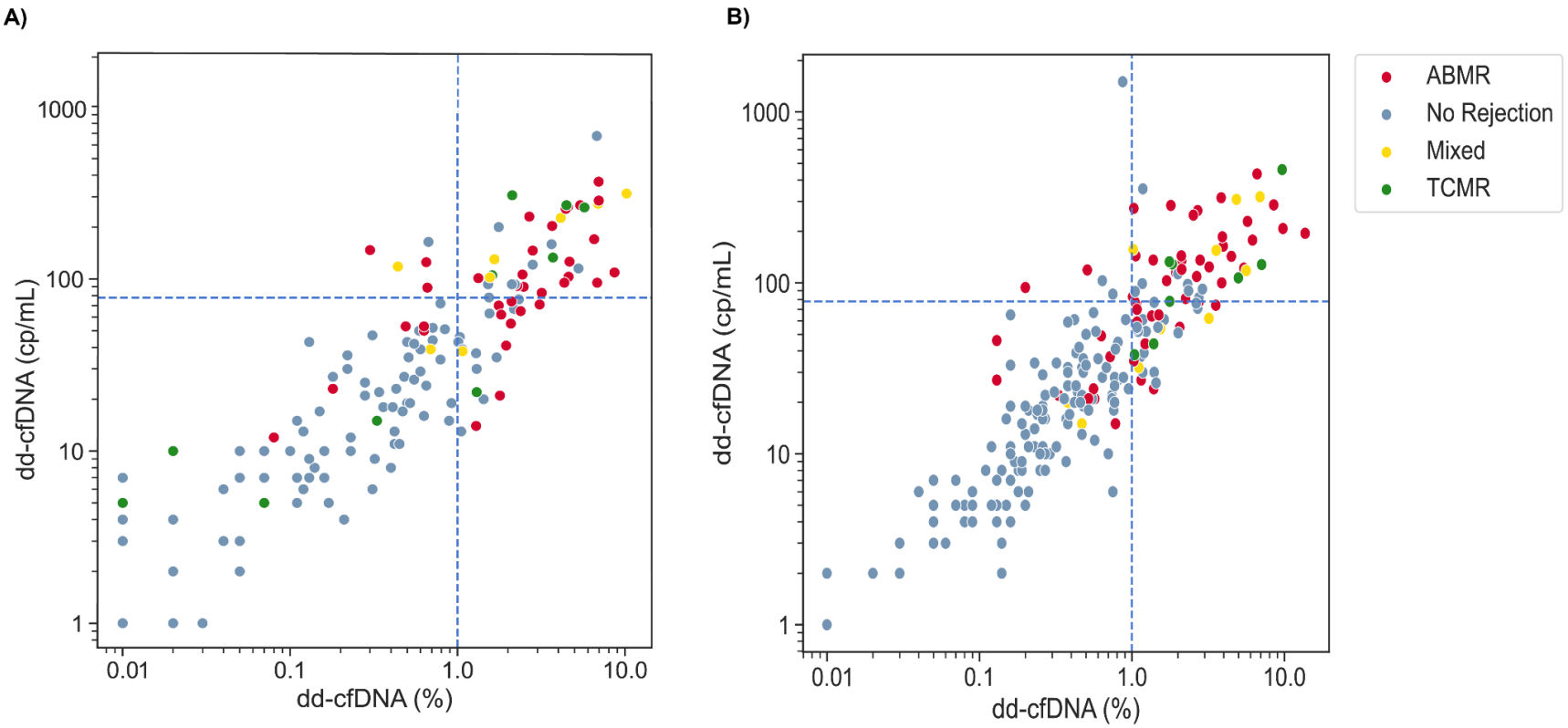

Figure S2: Plot of dd-cfDNA fraction (%) and quantity (cp/mL) based on MMDx for the training set (A, N=149) and test set (B, N= 218). The blue dashed horizontal and vertical lines indicate the dd-cfDNA quantity (78 cp/mL) and fraction (1%) thresholds, respectively. Patients with biopsy proven rejection: AMR, TCMR, Mixed, as adjudicated by MMDx, are depicted as red, green, and yellow dots, respectively. Patients with biopsies that show non-rejection are represented by gray dots. The two-threshold algorithm considers samples in the lower-left quadrant as low-risk for rejection, and samples in the remaining three quadrants, those with either dd-cfDNA quantity or fraction above the relevant thresholds, as high risk for rejection.

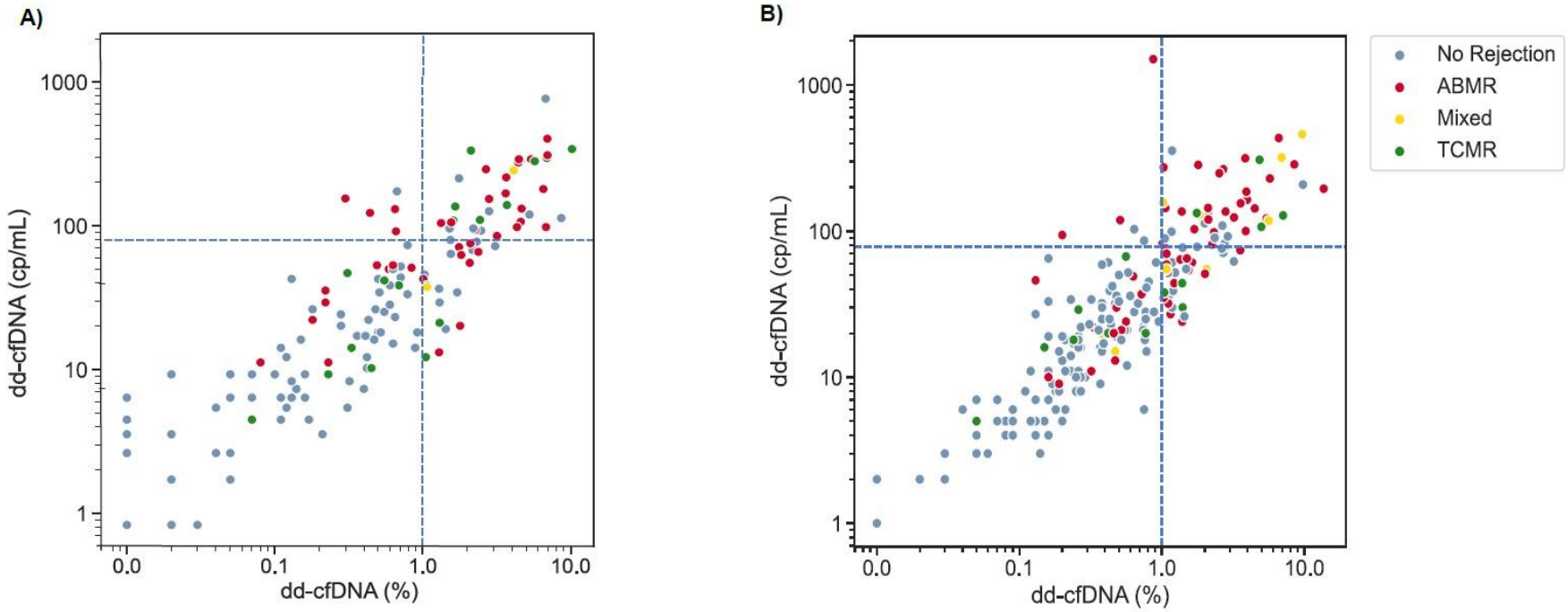

Figure S3: Plot of dd-cfDNA fraction (%) and quantity (cp/mL) based on histology (Banff criteria) for the training set (A, N=146) and test set (B, N=213). The blue dashed horizontal and vertical lines indicate the dd-cfDNA quantity (78 cp/mL) and fraction (1%) thresholds, respectively. Patients with biopsy proven rejection: AMR, TCMR, Mixed, as adjudicated by histology, are depicted as red, green, and yellow dots, respectively. Patients with biopsies that show non-rejection are represented by gray dots. The two-threshold algorithm considers samples in the lower-left quadrant as low-risk for rejection, and samples in the remaining three quadrants, those with either dd-cfDNA quantity or fraction above the relevant thresholds, as high risk for rejection.