Supplemental Digital Content

Table of Contents

Table S1: Performance of the dd-cfDNA fraction and quantity as determined by both MMDx and histology across total, training, and test sets ................................................................. 3

Table S2: Positive and negative predictive values for the two-threshold algorithm projected to different cohort AR prevalences, using molecular pathology as a comparator. .......... 4

Table S3: Published prospective studies assessing the performance of dd-cfDNA to detect rejection in renal allograft patients. ...................................................................................... 5

Figure S1 ....................................................................................................................... 6

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). ................................................................................. 7

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). ......................................................... 8
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.
