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# Cell-free DNA for the detection of kidney allograft rejection

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## Supplementary Information

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## 1 SUPPLEMENTARY TABLES

**Supplementary Table 1: Characteristics at the time of the biopsy with a concomitant dd-cfDNA in the development cohort**

|                                                              | <b>Derivation cohort<br/>(n=1,415)</b> |               |
|--------------------------------------------------------------|----------------------------------------|---------------|
|                                                              | <b>N</b>                               |               |
| <b>Estimated GFR, mean (SD)</b>                              | 1,415                                  | 47.65 (19.60) |
| <b>Proteinuria (g/g), mean (SD)</b>                          | 1,411                                  | 0.55 (1.39)   |
| <b>Mean fluorescence intensity of anti-HLA DSAs, No. (%)</b> | 1,400                                  |               |
| <b>&lt; 500</b>                                              |                                        | 861 (61.5)    |
| <b>500-3000</b>                                              |                                        | 426 (30.43)   |
| <b>3000 – 6000</b>                                           |                                        | 54 (3.86)     |
| <b>&gt; 6000</b>                                             |                                        | 59 (4.21)     |
| <b>Biopsy findings, No.(%)</b>                               | 1,415                                  |               |
| <b>Active AMR</b>                                            |                                        | 129 (9.12)    |
| <b>Chronic active AMR</b>                                    |                                        | 42 (2.97)     |
| <b>Inactive AMR</b>                                          |                                        | 11 (0.78)     |
| <b>Equivocal for diagnosis of AMR</b>                        |                                        | 5 (0.35)      |
| <b>Acute TCMR</b>                                            |                                        | 15 (1.06)     |
| <b>Chronic active TCMR</b>                                   |                                        | 19 (1.34)     |
| <b>Mixed rejection</b>                                       |                                        | 17 (1.20)     |
| <b>Borderline lesions</b>                                    |                                        | 19 (1.34)     |
| <b>Viral nephritis</b>                                       |                                        | 20 (1.41)     |
| <b>Glomerulitis without rejection</b>                        |                                        | 30 (2.12)     |
| <b>FSGS</b>                                                  |                                        | 48 (3.39)     |
| <b>IF-TA</b>                                                 |                                        | 557 (39.36)   |
| <b>No specific lesions</b>                                   |                                        | 503 (35.55)   |

**Supplementary Table 2: Baseline patient characteristics according to the presence or absence of rejection in the derivation cohort**

|                                                   | No rejection<br>(n=964) |               | Rejection<br>(n= 170) |               |
|---------------------------------------------------|-------------------------|---------------|-----------------------|---------------|
|                                                   | N                       |               | N                     |               |
| <b>Recipient characteristics</b>                  |                         |               |                       |               |
| <b>Age</b> (years), mean (SD)                     | 964                     | 52.30 (14.83) | 170                   | 51.79 (15.01) |
| <b>Sex male</b> , No. (%)                         | 964                     | 600 (62.24)   | 170                   | 93 (54.70)    |
| <b>Cause of end stage renal disease</b>           | 964                     |               | 170                   |               |
| <b>Glomerulopathy</b> , No. (%)                   |                         | 249 (25.83)   |                       | 45 (26.47)    |
| <b>Polycystic kidney disease</b> ,<br>No.(%)      |                         | 161 (16.70)   |                       | 15 (8.82)     |
| <b>Interstitial nephritis</b> (%)                 |                         | 77 (7.99)     |                       | 17 (10.00)    |
| <b>Diabetes</b> , No. (%)                         |                         | 90 (9.34)     |                       | 14 (8.24)     |
| <b>Vascular</b> , No. (%)                         |                         | 77 (7.99)     |                       | 16 (9.41)     |
| <b>Other</b> , No. (%)                            |                         | 122 (12.66)   |                       | 23 (13.53)    |
| <b>Unknown etiology</b> , No (%)                  |                         | 188 (19.50)   |                       | 40 (23.53)    |
| <b>Donor characteristics</b>                      |                         |               |                       |               |
| <b>Age</b> (years), mean (SD)                     | 960                     | 54.05 (15.91) | 169                   | 52.17 (19.65) |
| <b>Sex male</b> , No. (%)                         | 959                     | 501 (52.24)   | 168                   | 86 (51.19)    |
| <b>Deceased donor</b> , No. (%)                   | 963                     | 698 (72.48)   | 170                   | 122 (71.76)   |
| <b>Expanded criteria donor</b> , No. (%)          | 951                     | 333 (35.02)   | 169                   | 68 (40.24)    |
| <b>Transplant baseline characteristics</b>        |                         |               |                       |               |
| <b>Prior kidney transplant</b> , No. (%)          | 964                     | 141 (14.63)   | 170                   | 41 (24.12)    |
| <b>Cold ischemia time</b> (hours), mean (SD)      | 953                     | 14.23 (10.37) | 167                   | 15.88 (11.31) |
| <b>HLA-A/B/DR mismatch</b> , mean (SD), number    | 956                     | 3.53 (1.55)   | 166                   | 3.73 (1.37)   |
| <b>ABO incompatible transplantation</b> , No. (%) | 960                     | 32 (3.33)     | 166                   | 2 (1.20)      |

Abbreviations: SD, Standard deviation; HLA, human leukocyte antigen.

**Supplementary Table 3: Characteristics at the time of the biopsy with a concomitant dd-cfDNA in the validation cohort**

|                                       | <b>Validation cohort<br/>(n=2,317)</b> |             |
|---------------------------------------|----------------------------------------|-------------|
| <b>Biopsy findings, No.(%)</b>        | 2,317                                  |             |
| <b>AMR</b>                            |                                        | 352 (15.19) |
| <b>TCMR</b>                           |                                        | 224 (9.67)  |
| <b>Mixed rejection</b>                |                                        | 103 (4.45)  |
| <b>Borderline lesions</b>             |                                        | 183 (7.90)  |
| <b>Viral nephritis</b>                |                                        | 100 (4.32)  |
| <b>Glomerulitis without rejection</b> |                                        | 62 (2.68)   |
| <b>FSGS</b>                           |                                        | 32 (1.38)   |
| <b>IF-TA</b>                          |                                        | 624 (26.93) |
| <b>No specific lesions</b>            |                                        | 637 (27.49) |

**Supplementary Table 4: Baseline patient characteristics according to the presence or absence of rejection in the validation cohort**

|                                                   | <b>No rejection<br/>(n=1,252)</b> |               | <b>Rejection<br/>(n= 496)</b> |               |
|---------------------------------------------------|-----------------------------------|---------------|-------------------------------|---------------|
|                                                   | <b>N</b>                          |               | <b>N</b>                      |               |
| <b>Recipient characteristics</b>                  |                                   |               |                               |               |
| <b>Age</b> (years), mean (SD)                     | 1,249                             | 46.36 (18.41) | 496                           | 44.64 (17.41) |
| <b>Sex male</b> , No. (%)                         | 1,241                             | 751 (60.52)   | 494                           | 258 (52.23)   |
| <b>Cause of end stage renal disease</b>           | 1,224                             |               | 483                           |               |
| <b>Glomerulopathy</b> , No. (%)                   |                                   | 361 (29.49)   |                               | 148 (30.64)   |
| <b>Polycystic kidney disease</b> , No.(%)         |                                   | 145 (11.85)   |                               | 46 (9.52)     |
| <b>Interstitial nephritis</b> (%)                 |                                   | 145 (11.85)   |                               | 33 (6.83)     |
| <b>Diabetes</b> , No. (%)                         |                                   | 203 (16.58)   |                               | 87 (18.01)    |
| <b>Vascular</b> , No. (%)                         |                                   | 156 (12.75)   |                               | 79 (16.36)    |
| <b>Other</b> , No. (%)                            |                                   | 130 (10.62)   |                               | 71 (14.70)    |
| <b>Unknown etiology</b> , No (%)                  |                                   | 84 (6.86)     |                               | 19 (3.93)     |
| <b>Donor characteristics</b>                      |                                   |               |                               |               |
| <b>Age</b> (years), mean (SD)                     | 1,107                             | 44.14 (14.87) | 452                           | 39.38 (15.08) |
| <b>Sex male</b> , No. (%)                         | 910                               | 493 (54.18)   | 330                           | 161 (48.79)   |
| <b>Deceased donor</b> , No. (%)                   | 1,250                             | 939 (75.42)   | 489                           | 352 (71.26)   |
| <b>Expanded criteria donor</b> , No. (%)          | 1,125                             | 191 (16.98)   | 474                           | 75 (15.82)    |
| <b>Transplant baseline characteristics</b>        |                                   |               |                               |               |
| <b>Prior kidney transplant</b> , No. (%)          | 1,241                             | 155 (12.49)   | 491                           | 110 (22.40)   |
| <b>Cold ischemia time</b> (hours), mean (SD)      | 913                               | 13.09 (7.87)  | 347                           | 12.46 (8.52)  |
| <b>HLA-A/B/DR mismatch</b> , mean (SD), number    | 1,152                             | 3.80 (1.58)   | 458                           | 4.21 (0.81)   |
| <b>ABO incompatible transplantation</b> , No. (%) | 1,223                             | 16 (1.31)     | 481                           | 3 (0.62)      |

Abbreviations: SD, Standard deviation; HLA, human leukocyte antigen.

**Supplementary Table 5: Performance metrics of integrative dd-cfDNA in derivation and validation cohorts**

|                   | <b>Cut-off*</b> | <b>Sn</b> | <b>Sp</b> | <b>NPV</b> | <b>PPV</b> | <b>ROC<br/>AUC</b> | <b>PRAUC</b> | <b>Brier</b> |
|-------------------|-----------------|-----------|-----------|------------|------------|--------------------|--------------|--------------|
| Derivation cohort | 0.162           | 0.721     | 0.784     | 0.936      | 0.392      | 0.821              | 0.576        | 0.099        |
| Validation cohort | 0.239           | 0.769     | 0.768     | 0.887      | 0.584      | 0.838              | 0.671        | 0.149        |

\*Cut-off used to optimize the Youden index

Abbreviations: Sn, Sensitivity; Sp Specificity; NPV negative predictive value; PPV positive predictive value; SOC, standard of care

**Supplementary Table 6: Comparison of the performance metrics (sensitivity, specificity, positive predictive value, negative predictive value, ROC AUC, PRAUC, Brier score) of the model with dd-cfDNA and standard of care parameters, model with only standard of care parameters and dd-cfDNA alone when used as a continuous variable or with different thresholds in the derivation cohort.**

|                                        | Cut-off* | Sn    | Sp    | NPV   | PPV   | ROC AUC | PRAUC | Brier |
|----------------------------------------|----------|-------|-------|-------|-------|---------|-------|-------|
| Model with dd-cfDNA and SOC parameters | 0.162    | 0.721 | 0.784 | 0.936 | 0.392 | 0.821   | 0.576 | 0.099 |
| Model with only SOC parameters         | 0.165    | 0.677 | 0.780 | 0.926 | 0.373 | 0.777   | 0.478 | 0.111 |
| Dd-cfDNA continuous                    | 0.185    | 0.597 | 0.796 | 0.911 | 0.361 | 0.730   | 0.453 | 0.115 |
| Dd-cfDNA $\geq$ 0.5 %                  | 0.241    | 0.540 | 0.834 | 0.904 | 0.386 | 0.687   | 0.126 | 0.123 |
| Dd-cfDNA $\geq$ 1 %                    | 0.346    | 0.323 | 0.953 | 0.879 | 0.570 | 0.638   | 0.248 | 0.120 |

\*Cut-off used to optimize the Youden index

Abbreviations: Sn, Sensitivity; Sp Specificity; NPV negative predictive value; PPV positive predictive value; SOC, standard of care

**Supplementary Table 7: Comparison of the performance metrics (sensitivity, specificity, positive predictive value, negative predictive value, ROC AUC, PRAUC, Brier score) of the model with dd-cfDNA and standard of care parameters, model with only standard of care parameters and dd-cfDNA alone when used as a continuous variable or with different thresholds in the validation cohort.**

|                                        | Cut-off* | Sn    | Sp    | NPV   | PPV   | ROC AUC | PRAUC | Brier |
|----------------------------------------|----------|-------|-------|-------|-------|---------|-------|-------|
| Model with dd-cfDNA and SOC parameters | 0.294    | 0.762 | 0.777 | 0.885 | 0.591 | 0.842   | 0.676 | 0.145 |
| Model with only SOC parameters         | 0.340    | 0.558 | 0.835 | 0.817 | 0.588 | 0.743   | 0.575 | 0.172 |
| Dd-cfDNA continuous                    | 0.300    | 0.729 | 0.753 | 0.868 | 0.555 | 0.795   | 0.586 | 0.164 |
| Dd-cfDNA $\geq 0.5$ %                  | 0.320    | 0.768 | 0.695 | 0.876 | 0.516 | 0.732   | 0.094 | 0.172 |
| Dd-cfDNA $\geq 1$ %                    | 0.383    | 0.590 | 0.832 | 0.828 | 0.598 | 0.711   | 0.184 | 0.172 |

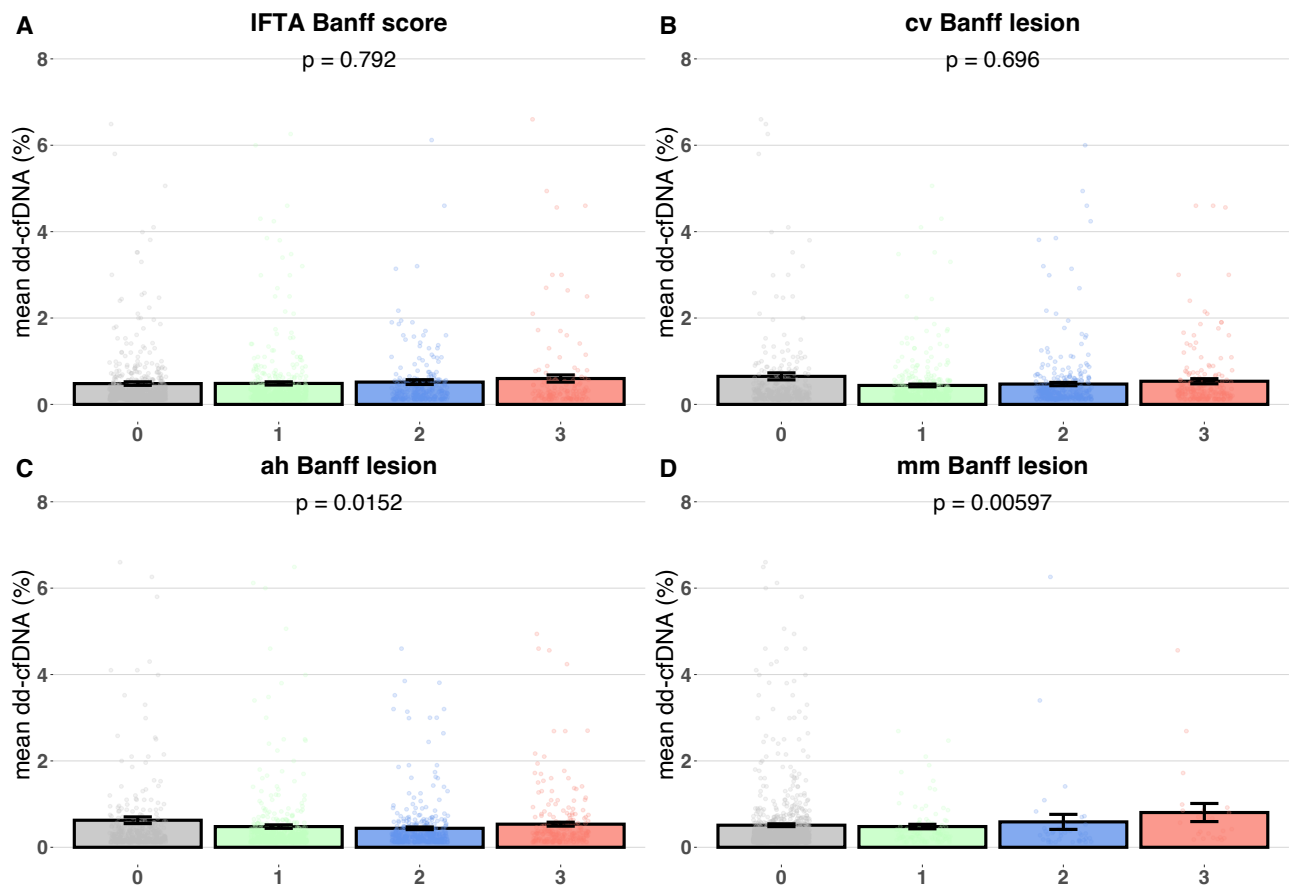
\*Cut-off used to optimize the Youden index

Abbreviations: Sn, Sensitivity; Sp Specificity; NPV negative predictive value; PPV positive predictive value; SOC, standard of care

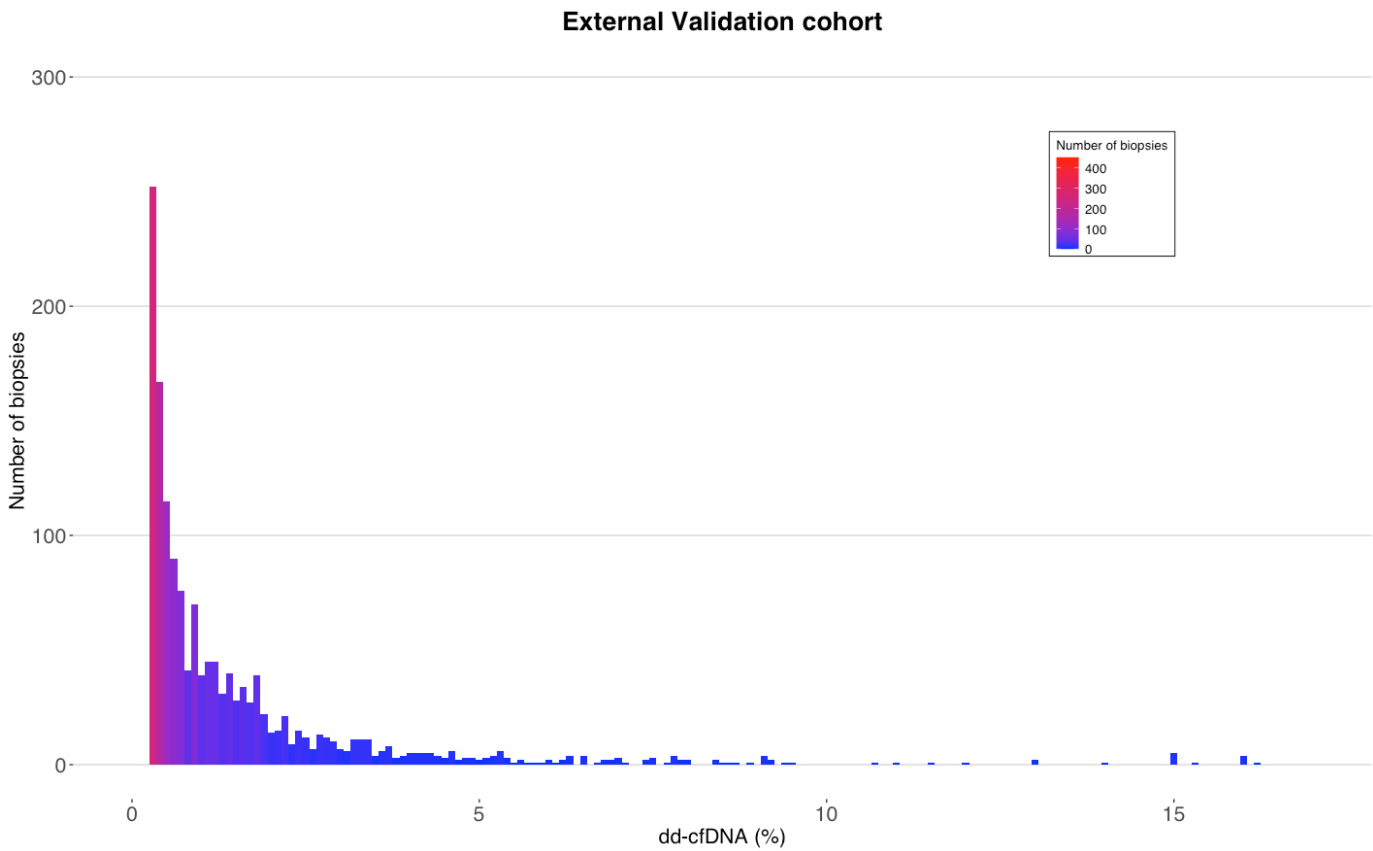
## 2 SUPPLEMENTARY FIGURES

### Supplementary Figure 1: Association of dd-cfDNA with Chronic Banff score IFTA (interstitial fibrosis and tubular atrophy), cv Banff score (arteriosclerosis), ah Banff score (arteriolar hyalinosis), and mm Banff score (mesangial expansion)

This figure shows the mean levels of dd-cfDNA according to the chronic Banff score IFTA (interstitial fibrosis and tubular atrophy) (Panel A), cv Banff score (arteriosclerosis) (Panel B), ah Banff score (arteriolar hyalinosis) (Panel C), and mm Banff score (mesangial expansion) (Panel E). Each of these scores ranges from 0 to 3, with higher scores indicating more severe lesions. The lesions were defined according to Banff 2019 classification. Each dot corresponds to an individual dd-cfDNA value. Data are presented as mean values  $\pm$  SEM. Comparisons between the groups was performed using two-sided Kruskal-Wallis test with adjustments for multiple comparisons. This figure shows the absence of increment of dd-cfDNA with the severity of the lesions.

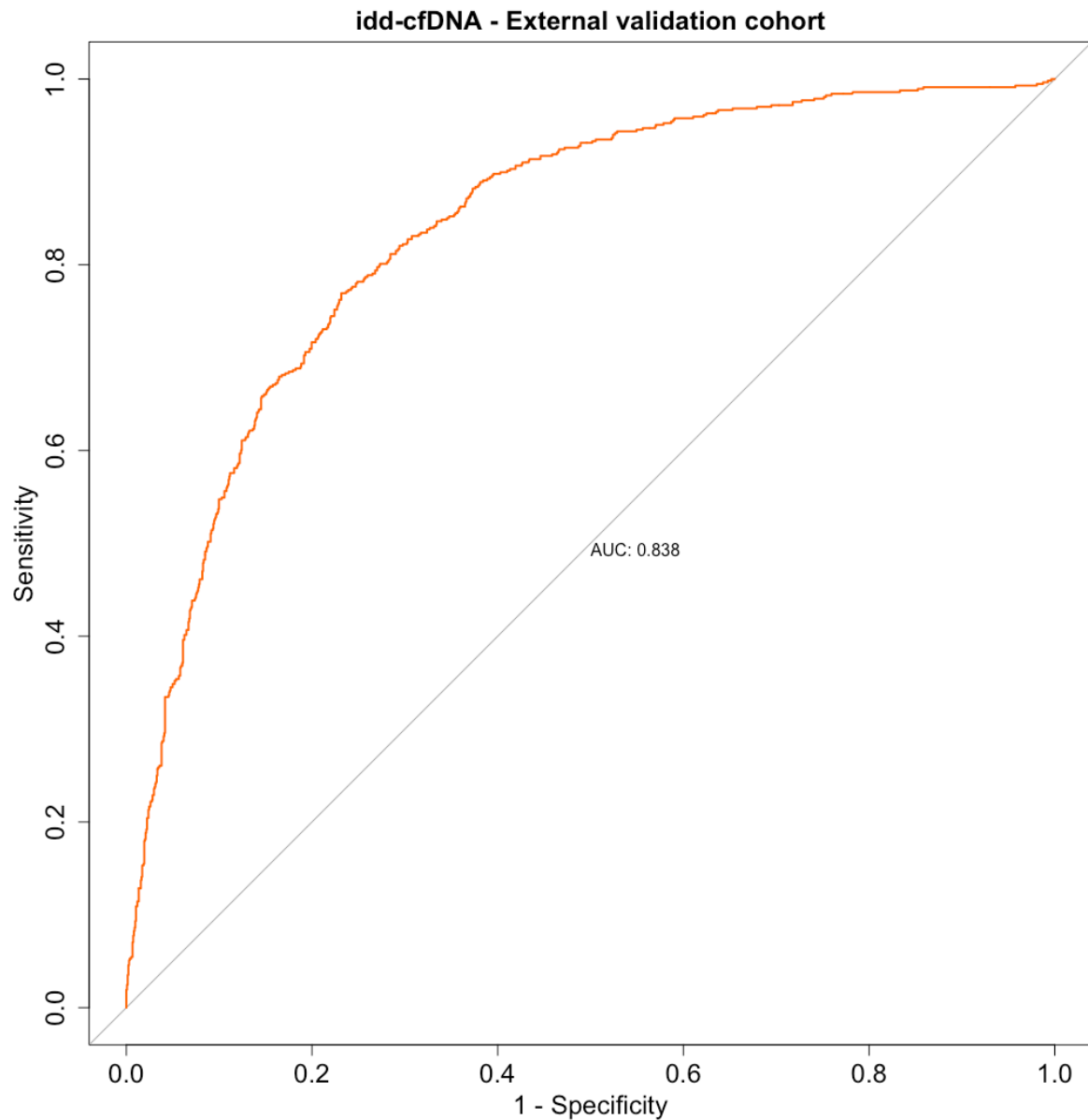


**Supplementary Figure 2: Distribution of the dd-cfDNA in the validation cohort**  
This figure shows the distribution of dd-cfDNA in the validation cohort.



**Supplementary Figure 3: ROC curve of the integrative dd-cfDNA score in the validation cohort**

This figure shows the receiver-operating (ROC) curve of the integrative dd-cfDNA score in the validation cohort. The area under the curve of the score is equal to 0.838 (bias-corrected 95% CI: 0.817 – 0.855), indicating a good discrimination.



**Supplementary Figure 4: Decision Curve Analysis evaluating the clinical utility of the addition of dd-cfDNA in Predicting rejection compared to standard of care parameters.**

The vertical axis represents the net benefit, which is a measure of the overall impact of the model on decision-making. The horizontal axis represents the threshold probability, which is the risk level that would prompt a biopsy. The red line corresponds to the assumption that all patients will have a biopsy, the green line that no patients will have a biopsy, the purple line to the decision of performing a biopsy based on the standard of care parameters, and the blue line to the decision of performing a biopsy based on the standard of care parameters with the addition of dd-cfDNA. This figure shows that the net benefit for decisions on the basis of the model integrating dd-cfDNA is higher than that for decisions on the basis the standard of care model.

