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# Donor-derived Cell-free DNA Shows High Sensitivity for the Diagnosis of Pancreas Graft Rejection in Simultaneous Pancreas-kidney Transplantation

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**Background.** Pancreas graft status in simultaneous pancreas-kidney transplant (SPKTx) is currently assessed by nonspecific biochemical markers, typically amylase or lipase. Identifying a noninvasive biomarker with good sensitivity in detecting early pancreas graft rejection could improve SPKTx management. **Methods.** Here, we developed a pilot study to explore donor-derived cell-free DNA (dd-cfDNA) performance in predicting biopsy-proven acute rejection (P-BPAR) of the pancreas graft in a cohort of 36 SPKTx recipients with biopsy-matched plasma samples. dd-cfDNA was measured using the Prospera test (Natera, Inc.) and reported both as a fraction of the total cfDNA (fraction; %) and as concentration in the recipient's plasma (quantity; copies/mL). **Results.** In the absence of P-BPAR, dd-cfDNA was significantly higher in samples collected within the first 45 d after SPKTx compared with those measured afterward (median, 1.00% versus 0.30%; median, 128.2 versus 35.3 cp/mL, respectively with both;  $P=0.001$ ). In samples obtained beyond day 45, P-BPAR samples presented a significantly higher dd-cfDNA fraction (0.83 versus 0.30%;  $P=0.006$ ) and quantity (81.3 versus 35.3 cp/mL;  $P=0.001$ ) than stable samples. Incorporating dd-cfDNA quantity along with dd-cfDNA fraction outperformed dd-cfDNA fraction alone to detect active rejection. Notably, when using a quantity cutoff of 70 cp/mL, dd-cfDNA detected P-BPAR with a sensitivity of 85.7% and a specificity of 93.7%, which was more accurate than current biomarkers (area under curve of 0.89 for dd-cfDNA (cp/ml) compared with 0.74 of lipase and 0.46 for amylase). **Conclusions.** dd-cfDNA measurement through a simple noninvasive blood test could be incorporated into clinical practice to help inform graft management in SPKTx patients.

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## INTRODUCTION

Simultaneous pancreas-kidney transplant (SPKTx) is considered the best treatment alternative for patients with type 1 diabetes (T1D) and end-stage renal disease (ESRD).<sup>1-4</sup> Diabetic nephropathy is a microvascular complication caused by sustained hyperglycemia and is one of the leading causes of ESRD.<sup>2</sup> SPKTx can significantly improve prognosis and health status in patients with insulin-dependent diabetes as it can reestablish euglycemia and thus lead to a reduction of the predicted risk for microvascular and macrovascular complications.<sup>5,6</sup>

Pancreas graft rejection is a leading cause of graft failure,<sup>7,8</sup> with acute rejection incidences of up to 21% in the first 1 y.<sup>8-11</sup> Current tools for assessing graft rejection rely on clinical laboratory tests that evaluate the exocrine (eg, amylase, lipase) or endocrine (eg, glycemia, hemoglobin A1c, C-peptide) functionality of the graft. Remarkably, these tests are highly unspecific because the native pancreas' exocrine function is preserved in most patients, and hence elevation in any of these parameters may not be related to pancreas graft rejection. Pancreas graft biopsy is the gold standard for the diagnosis of acute rejection. However, biopsies are an invasive procedure with a significant rate of complications<sup>12</sup> and often cannot be performed<sup>13</sup> or provide no significant information (up to 39% of the time) despite the presence of graft dysfunction.<sup>11</sup> Therefore, a clear need exists for a noninvasive, donor-specific, dynamic biomarker to assess allograft status and monitor for injury/rejection that can ultimately improve management in SPKTx recipients.

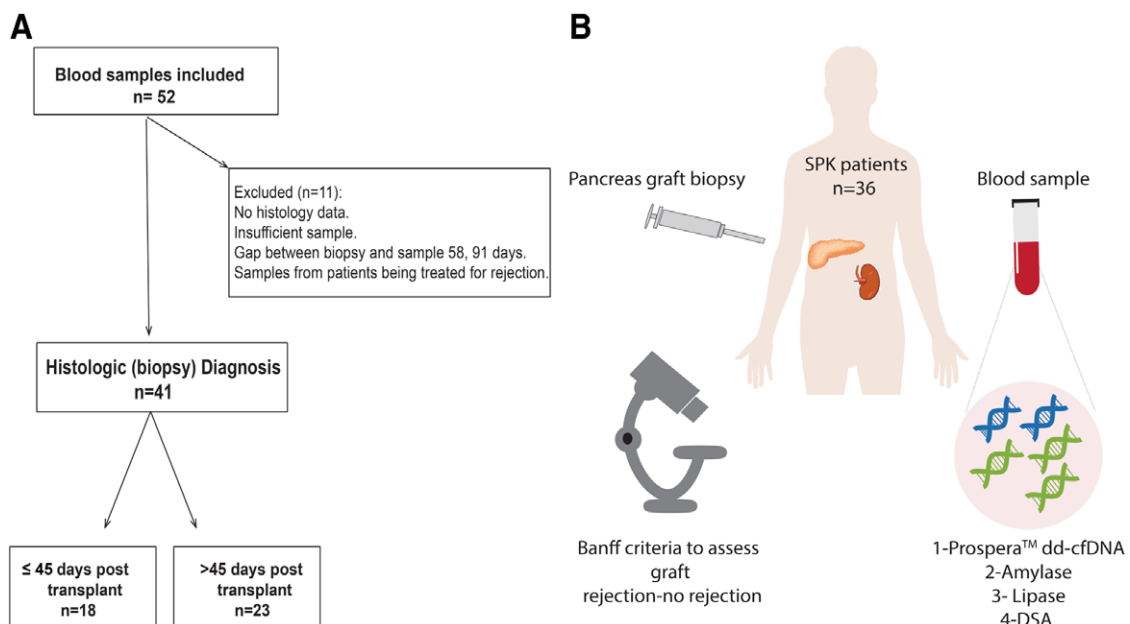
Several studies have demonstrated that measurement of donor-derived cell-free DNA (dd-cfDNA) in the blood of recipients of solid organ transplants (lung, kidney, heart, liver) can distinguish the risk of allograft rejection from nonrejection.<sup>14-19</sup> Studies evaluating the potential of

dd-cfDNA for assessing risk of rejection in SPKTx recipients are limited.<sup>20</sup> In this pilot study, we evaluated the performance of the Prospera test,<sup>21</sup> which uses a single-nucleotide polymorphism-based massively multiplexed polymerase chain reaction (PCR) methodology to measure both dd-cfDNA fraction and quantity, to assess risk of rejection in 36 SPKTx recipients who were histologically profiled for graft status.

## MATERIALS AND METHODS

### Study Design and Patient's Population

This was a retrospective study conducted between April 2017 and February 2021 and included 36 patients with T1D and ESRD who received an SPKTx and were admitted for pancreas graft biopsy (either per protocol or for-cause) at Hospital Clinic Barcelona, Spain. Eleven out of 52 blood samples were excluded from the study because of (1) lack of paired histology data, (2) insufficient sample for biopsy, (3) gap between biopsy and blood collection dates of >30 d, and (4) samples obtained while a patient was already undergoing treatment for graft rejection (Figure 1A). The remaining pancreas biopsy-paired plasma samples (n=41) were included in the analysis. To account for the possible influence of donor-related and immediate postoperative complications on dd-cfDNA quantification, we considered samples collected before and after 45 d after SPKTx separately. Out of the total 41 graft biopsies, 18 were collected ≤45 d post-transplant, and 23 were collected >45 d posttransplant. The study was approved by the local ethical institutional review board (HCB\_2016\_0479) and was conducted in full adherence to the Declaration of Helsinki. All patients provided written informed consent to participate in the research.



**FIGURE 1.** A, A flowchart showing sample exclusion criteria used for this study. B, Schematic of experimental design. SPKTx recipients (n=36) were admitted for biopsy. Pancreatic biopsy was used to classify graft rejection vs no rejection per Banff criteria. Blood samples, collected from patients at the time of biopsy, were used to assess (1) both dd-cfDNA fraction and quantity using the Prospera test, (2) amylase, (3) lipase, and (4) DSAs. dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; SPKTx, simultaneous pancreas-kidney transplant.

## Patient's Samples

Pancreas graft biopsies were performed either per protocol or for-cause. As per center protocol, for-cause biopsies were indicated if patients presented a persistent ( $\geq 2$  determinations separated  $>48$  h apart) elevation ( $\geq 2\times$  normal value) in pancreatic enzymes (amylase and/or lipase). Samples were obtained by ultrasound-guided percutaneous needle punch and classified according to the 2011 Banff criteria.<sup>22</sup> For analysis purposes, biopsies were further reclassified as “nonrejection” or “rejection,” the latter including Banff categories: indeterminate, T cell–mediated rejection, and antibody-mediated rejection (ABMR). Whole blood and serum samples were obtained on the day of pancreas graft biopsy, before the performance of biopsy, to avoid misleading interpretation of dd-cfDNA.<sup>23</sup> Whole blood samples were used to measure dd-cfDNA levels, whereas serum samples were used to measure amylase (U/L), lipase (U/L), and creatinine (mg/dL) (Figure 1B). In addition, serum samples were screened for HLA<sup>23</sup> class I and II donor-specific antibodies (DSAs) using the Lifecodes LifeScreen Deluxe flow bead assay (Immucor, Stamford, CT). Antibody specificities were determined using the Lifecodes Single Antigen bead assay (Immucor) in patients with positive screening for HLA antibodies. The DSAs were considered positive with mean fluorescence intensity of  $>1500$  according to the protocols of the Histocompatibility Laboratory of Catalunya. A/B/DRB1 HLA loci were considered for DSA in all patients, whereas DQB1/DP1/C HLA loci were considered for DSAs only when they were available.

## Assessment of dd-cfDNA Levels Using the Prospera Test

Whole blood was drawn into PAXgene blood cfDNA DNA tubes (QIAGEN), and plasma samples were obtained through double centrifugation of whole blood following the manufacturer's instructions. Plasma was then stored at  $-80$  °C until sample processing. Massively multiplexed PCR was used to amplify cfDNA in plasma samples, targeting 13926 single-nucleotide polymorphisms, followed by sequencing of amplicons (The Prospera test, Natera Inc., Austin, TX) as described previously.<sup>21</sup> Samples were run according to standard the Clinical Laboratory Improvement Amendments of 1988 (CLIA) protocol, except for samples with  $<4$  mL plasma that had 9 additional PCR cycles. dd-cfDNA levels were reported as a fraction of the total cfDNA (%; median [interquartile range, IQR]) and as a concentration in the recipient's plasma (copies/mL; median [IQR]).

## Statistical Analysis

Comparisons of median measurements were performed using the Mann–Whitney U test and  $P$  value of  $<0.05$  was considered statistically significant. When needed,  $P$  values were adjusted for multiple testing using Benjamini–Hochberg adjustment. Receiver operating characteristic curves (ROC) were constructed, and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for various thresholds. Statistical analyses were performed in Python programming language using SciPy and statsmodels packages (Python V.3.6 Software Foundation, version; <https://www.python.org/psf>). Graphical representation of continuous variables is shown as median (IQR).

## RESULTS

### Patients' Demographics/Characteristics

Between April 2017 and February 2021, a total of 41 biopsy-matched from 36 SPKTx patients receiving care at the Hospital Clinic Barcelona were included in this study, with a mean age of 42.3 y, a median weight of 58.7 kg (IQR, 54–66), and body mass index of  $22.0 \text{ kg/m}^2$  (IQR, 20–23). A total of 18 acute rejection episodes were diagnosed (43%), of which 6 (33%) were indeterminate, 9 (50%) were T cell–mediated rejection, and 3 (17%) were ABMR, with a median time to graft rejection of 147 d (IQR, 27–400). Figure S1 (SDC, <http://links.lww.com/TP/C374>) shows the duration in days between the transplant and biopsy for samples performed per protocol or for-cause, and Table 1 shows the detailed demographic and immunologic data of the study cohort.

### dd-cfDNA and Pancreas Graft Rejection

The median dd-cfDNA fraction was significantly higher in patients with biopsy-proven acute rejection (P-BPAR) of pancreatic graft (1.05% [0.81–1.67]), compared with those with nonrejection (0.52% [IQR, 0.21–0.78];  $P=0.0004$ ; Figure 2A). Similarly, the median absolute dd-cfDNA quantity was significantly higher in patients with P-BPAR (103.70 cp/mL [IQR, 76.70–189.80]) compared with those with nonrejection (51.5 cp/mL [IQR, 22.2–76.7];  $P=0.0007$ ; Figure 2B). These data suggest that both dd-cfDNA fraction and quantity can discriminate between pancreatic graft rejection and nonrejection status in SPKTx recipients.

To explore the potential confounding factor of donor and surgery-associated organ injury, we compared the dd-cfDNA levels before and after 45 d posttransplant (Figure 3). Table 2 shows the baseline data for the patients with samples collected  $>45$  posttransplant ( $n=23$ ). In patients with no rejection, the fraction of dd-cfDNA and absolute quantity of dd-cfDNA were significantly higher in the early postoperative period compared with those with biopsy performed after day 45 (median, 1.00% versus 0.30%;  $P=0.001$ ; median, 128.2 versus 35.3 cp/mL;  $P=0.001$ ). During the first 45 d after SPKTx, there were no statistical differences in dd-cfDNA levels between nonrejection samples and those with BPAR, either as a fraction of dd-cfDNA ( $P=0.120$ ; Figure 3A) or as dd-cfDNA quantity ( $P=0.290$ ; Figure 3B). In contrast, in biopsy-matched blood samples collected  $>45$  d posttransplant, both dd-cfDNA fraction and dd-cfDNA quantity were significantly higher in the BPAR cohort (0.83% [IQR, 0.67–1.58]; 81.3 cp/mL [IQR, 73.4–152.0]) compared with the nonrejection cohort (0.30% [IQR, 0.14–0.52],  $P=0.006$ , and 35.3 cp/mL [IQR, 19.5–55.0],  $P=0.001$ , respectively; Figure 3). When excluding indeterminate biopsies from the acute rejection group, dd-cfDNA levels were still significantly elevated compared with nonrejection cases (0.81% [0.52–0.83] rejection versus 0.30% [0.14–0.52] nonrejection;  $P=0.031$ ). These data suggest that both dd-cfDNA fraction and quantity can distinguish between graft rejection and nonrejection status after 45 d posttransplant.

We next aimed to identify an optimum cutoff value of dd-cfDNA that would accurately discriminate pancreatic graft rejection from nonrejection. We assessed the ability of 2 recently published thresholds in detecting kidney allograft

**TABLE 1.****Study demographics and baseline characteristics of the study cohort**

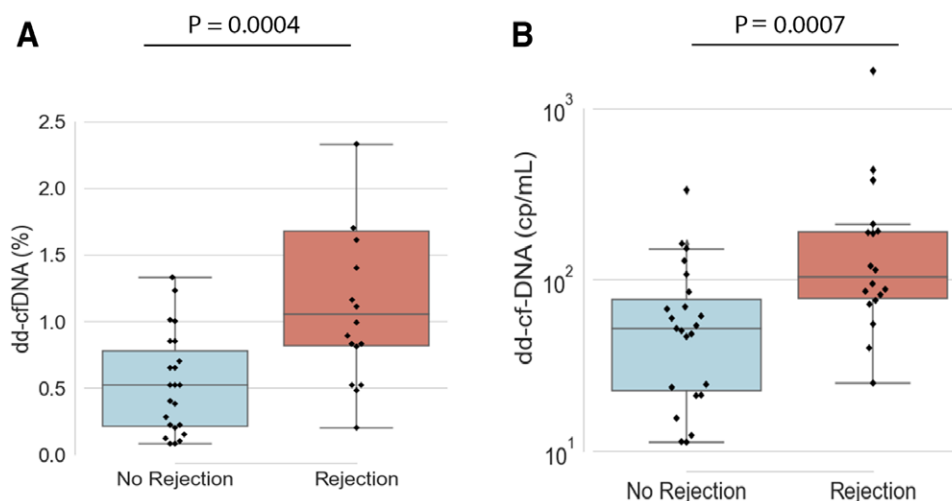
SPKTx samples	All (N = 41)	Rejection (N = 18)	Nonrejection (N = 23)
Age (y), median (IQR)	42.3 (33.5–48.3)	38.4 (31.9–47.5)	43.7 (35.8–50.3)
Gender (M/F)	21/20	10/8	11/12
Diabetes type	T1D: 39 T2D: 2	T1D: 18 T2D: 0	T1D: 21 T2D: 2
Diabetes vintage (y), median (IQR)	26.1 (22.0–32.9)	26.3 (22.3–30.2)	25.0 (19.5–33.04)
Weight (kg), median (IQR)	58.7 (54.5–66.0)	57.8 (54.9–64.2)	62.5 (54.9–68.3)
BMI (Kg/m <sup>2</sup> ), median (IQR)	22.03 (20.1–23.73)	22.03 (21.3–24.2)	22.0 (19.6–24.2)
Pancreas biopsy date	≤45 d posttransplant (n = 18) >45 d posttransplant (n = 23)	≤45 d posttransplant (n = 11) >45 d posttransplant (n = 7)	≤45 d posttransplant (n = 7) >45 d posttransplant (n = 16)
Amylase (U/L), median (IQR)	99.0 (75.0–142.0)	100 (76.50–178.7)	96.0 (77.5–138.0)
Lipase (U/L), median (IQR)	50.0 (35.0–118.0)	102.00 (57.25–131.75)	42.0 (32.0–55.0)
Creatinine (mg/dL), median (IQR)	1.06 (0.87–1.41)	1.02 (0.855–1.43)	1.11 (0.91–1.40)
cPRA, median (IQR)	39.0 (0.0–51.0)	48.0 (17.5–52.5)	0.5 (0.0–47.5)
HLA mismatches (ABDR)	1/6: 0 2/6: 2 3/6: 3 4/6: 7 5/6: 17 6/6: 8 No data: 4	1/6: 0 2/6: 0 3/6: 1 4/6: 4 5/6: 10 6/6: 3	1/6: 0 2/6: 2 3/6: 2 4/6: 3 5/6: 7 6/6: 5 No data: 4
Reason for biopsy	For-cause: 13 Protocol: 28	For-cause: 8 Protocol: 10	For-cause: 5 Protocol: 18

ABDR, HLA- A, HLA-B, HLA-C, and HLA-DRB1; BMI, body mass index; cPRA, calculated panel reactive antibodies; IQR, interquartile range; M/F, male/female; SPKTx, simultaneous pancreas-kidney transplant; T1D, type 1 diabetes; T2D, type 2 diabetes.

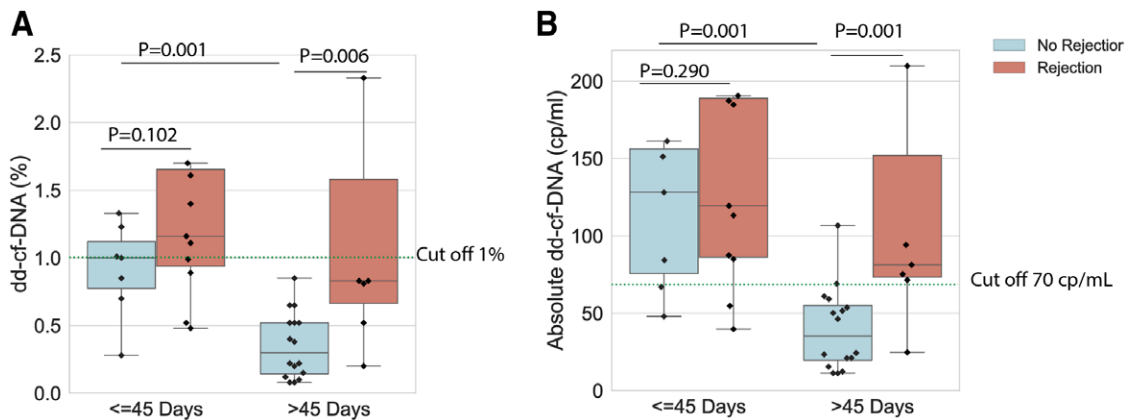
rejection by applying (1) a cutoff of 1% dd-cfDNA,<sup>14</sup> and (2) a 2-threshold algorithm that combined the dd-cfDNA fraction cutoff ( $\geq 1\%$ ) and a dd-cfDNA quantity cutoff of  $\geq 78$  cp/mL.<sup>24</sup> Sensitivity using dd-cfDNA fraction alone was 28.6% (2/7). Sensitivity was considerably higher, at 57.1% (4/7), when using the 2-threshold algorithm that combines dd-cfDNA fraction and dd-cfDNA quantity. Specificity was excellent for both cutoffs, at 100% and 93.7%, respectively. When using a dd-cfDNA quantity cutoff value of 70 cp/mL (green dotted line in Figure 3B) the sensitivity increased to 85.7% (6/7) while maintaining a high specificity of 93.7%, along with a PPV of 85.7% and NPV of 93.7%.

**dd-cfDNA and DSA**

Although only 1 of the biopsies collected >45 d post-transplant was characterized with ABMR, 3 patients were found to have circulating DSAs. We found that dd-cfDNA fraction was significantly elevated in samples tested positive for DSAs (0.83% [0.82–2.5]) versus those tested negative (0.39% [0.18–0.55];  $P = 0.022$ ; Figure 4A). Similarly, we found that dd-cfDNA quantity was significantly elevated in samples tested positive for DSAs (94.2 cp/mL [84.7–264.1]) versus those tested negative (48.1 cp/mL [21.0–63.0];  $P = 0.024$ ; Figure 4B).



**FIGURE 2.** Comparison of median dd-cfDNA fractions (%) (A) and quantity (cp/mL) (B) between biopsy-proven pancreas rejection (n = 18) and no rejection (n = 23) groups. Data are presented as median (IQR). dd-cfDNA, donor-derived cell-free DNA; IQR, interquartile range.



**FIGURE 3.** Comparison of median dd-cfDNA levels of (A) fraction (%) and (B) quantity (cp/mL) between biopsy-proven pancreas rejection and no rejection groups before and after 45 d posttransplant. Biopsy-proven pancreas rejection samples before and after 45 d were 11 and 7, respectively. Biopsies with no rejection samples before and after 45 d were 7 and 16, respectively. Data are presented as median (IQR). dd-cfDNA, donor-derived cell-free DNA; IQR, interquartile range.

### dd-cfDNA Performance Compared With Other Biomarkers

Next, we sought to compare the performance of dd-cfDNA with conventional clinical tests used in assessing graft surveillance. We measured amylase and lipase levels in blood samples drawn concurrently with pancreatic biopsies. Although amylase levels did not significantly change between rejection and nonrejection groups ( $P=0.40$ ; Figure 5A), lipase was significantly higher in the rejection group compared with nonrejection ( $P=0.038$ ; Figure 5B). We compared the diagnostic ability of amylase, lipase, and dd-cfDNA (fraction and quantity) in distinguishing graft rejection from nonrejection based on histopathology results of pancreas grafts biopsies >45 d posttransplant. The calculated area under curve values (Figure 5C) of these biomarkers in discriminating pancreatic graft rejection from nonrejection were as follows: dd-cfDNA quantity: 0.89; dd-cfDNA fraction: 0.84; lipase: 0.74; and

amylase: 0.46. These data suggest that dd-cfDNA is superior to the marker assays traditionally used to discriminate pancreas rejection from nonrejection in SPKTx recipients. It is noteworthy to mention that attempts to combine dd-cfDNA and lipase simultaneously did not enhance the performance of dd-cfDNA.

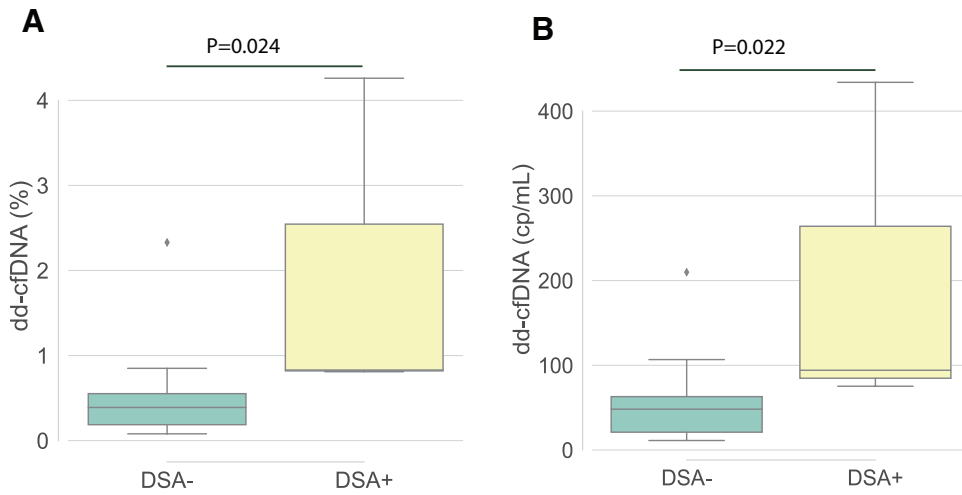
### DISCUSSION

This pilot study explored, for the first time, the performance of dd-cfDNA to diagnose pancreas graft rejection in SPKTx recipients. We found that among stable patients, dd-cfDNA levels were elevated during the first 45 d after transplantation, compared with those performed after day 45. During this early period ( $\leq 45$  d), dd-cfDNA could not discriminate between P-BPAR and nonrejection. Of relevance, in biopsies performed >45 d posttransplant, dd-cfDNA quantity could discriminate between those with P-BPAR and those without acute rejection, with a sensitivity

**TABLE 2.** Study demographics and baseline characteristics for >45 d posttransplant

Parameter	Overall (N = 23)	Rejection group (N = 7)	No rejection group (N = 16)
Age (y), median (IQR)	42.9 (32.7–48.7)	33.7 (30.5–42.6)	43.9 (35.8–50.3)
Gender (M/F)	11/12	2/5	9/7
Weight (kg), median (IQR)	58.7 (56.05–69.10)	57.5 (55.4–64.4)	63.6 (57.4–69.0)
BMI (Kg/m <sup>2</sup> )	22.4 (20.7–24.1)	22.4 (21.5–23.1)	22.6 (20.2–24.2)
Diabetes type	T1D: 22 T2D: 1	T1D: 7 T2D: 0	T1D: 15 T2D: 1
Diabetes vintage (y)	26.4 (18.7–32.3)	23.4 (20.3–28.5)	26.7 (18.6–33.0)
cPRA at transplant, median (IQR)	0.0 (0.0–47.0)	43.0 (26.5–60.5)	0.0 (0.0–23.5)
HLA mismatches (ABDR)	1/6: 0 2/6: 1 3/6: 1 4/6: 5 5/6: 9 6/6: 5 No data: 2	1/6: 0 2/6: 0 3/6: 0 4/6: 2 5/6: 4 6/6: 1	1/6: 0 2/6: 1 3/6: 1 4/6: 3 5/6: 5 6/6: 4 No data: 2
Reason for biopsy	For-cause: 6 Protocol: 17	For-cause: 3 Protocol: 4	For-cause: 3 Protocol: 13
Creatinine (mg/dL), median (IQR)	1.1 (0.9–1.52)	0.9 (0.8–1.7)	1.2 (1.0–1.4)

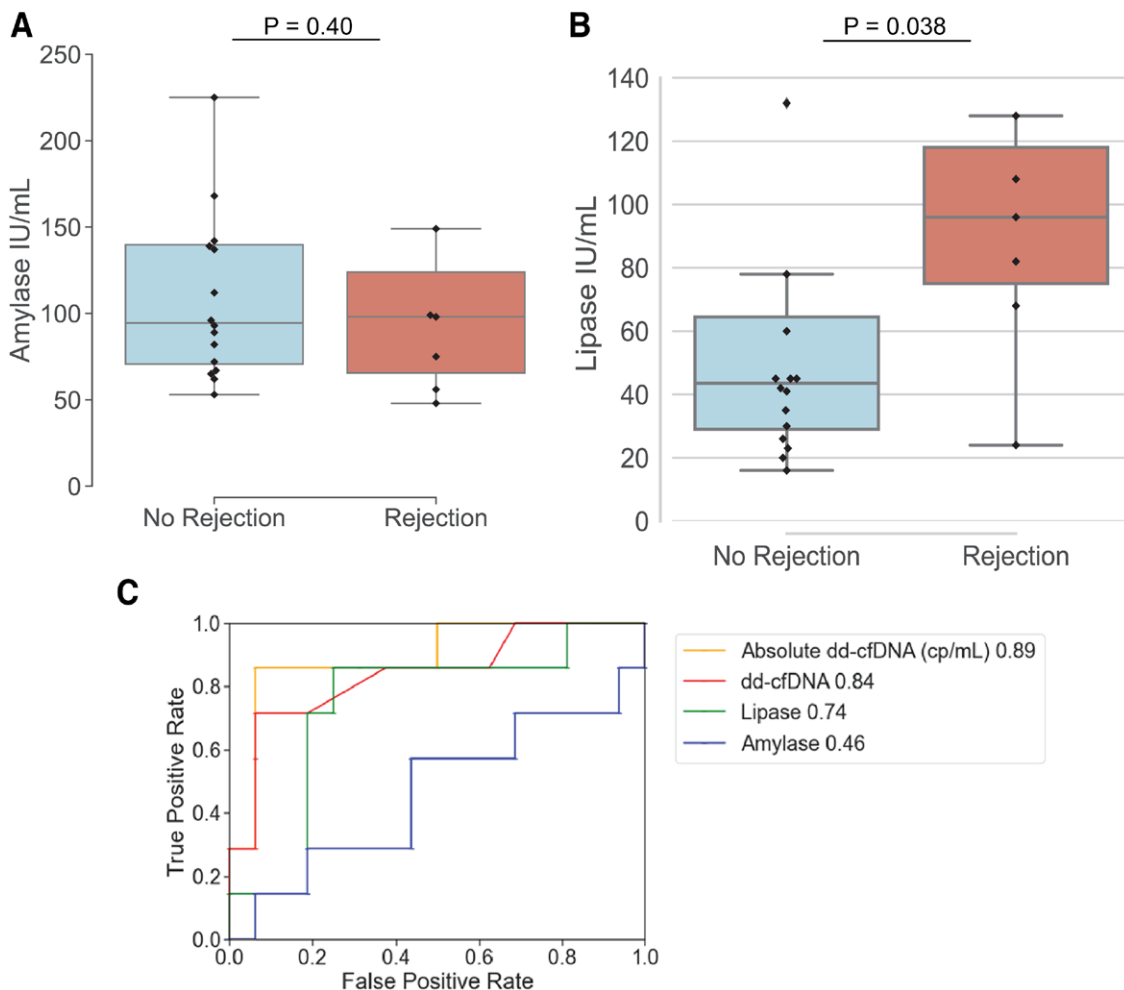
ABDR, HLA-A, HLA-B, HLA-C, HLA-C, and HLA-DRB1; BMI, body mass index; cPRA, calculated panel reactive antibodies; IQR, interquartile range; M/F, male/female; T1D, type 1 diabetes; T2D, type 2 diabetes.



**FIGURE 4.** Median dd-cfDNA fraction (%) (A) and quantity (cp/mL) (B) in samples positive for DSAs (DSA+) vs no DSAs (DSA-) in >45 d samples. Data are presented as median (IQR). dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; IQR, interquartile range.

and specificity of 85% and 93%, respectively. Moreover, dd-cfDNA demonstrated better performance than the currently available biomarkers, amylase and lipase. Of note, combining lipase and dd-cfDNA did not increase diagnostic accuracy compared with dd-cfDNA alone.

Pancreas graft rejection is a leading cause of graft dysfunction and graft failure, with incidences during the first year up to 21%.<sup>8,9</sup> The diagnosis of graft rejection is challenging because biomarkers used in clinical practice (amylase and lipase) are also expressed by patients' native pancreas and



**FIGURE 5.** Median levels of (A) amylase ( $P = 0.40$ ) and (B) lipases ( $P = 0.038$ ) in samples collected >45 d posttransplant. Data are presented as median (IQR). C, ROC curve for dd-cfDNA fraction (%), quantity (cp/mL), amylase and lipase, and corresponding AUC values. AUC, area under the curve; dd-cfDNA, donor-derived cell-free DNA; IQR, interquartile range; ROC, receiver operating characteristic.

may cause an incorrect diagnosis of rejection. In biopsies performed in SPKTx recipients with pancreas graft dysfunction, the incidence of P-BPAR has been observed to be as high as 86%.<sup>11</sup> The high observed correlation between lipase levels and P-BPAR in our study may be related to a positive selection bias of biopsies performed for-cause, in which elevation of lipase levels was the major criteria for performing pancreas graft biopsy. Nonetheless, dd-cfDNA performance was superior to lipase in diagnosing P-BPAR, with an area under curve of up to 0.89. In the future, it would be interesting to evaluate whether longitudinal quantification of dd-cfDNA can predict a pancreas graft acute rejection episodes in advance of the elevation of clinical biomarkers, as has been shown in kidney and liver transplantation.<sup>25,26</sup> As Uva et al<sup>11</sup> demonstrated, in SPKTx patients with pancreas graft dysfunction but without kidney acute rejection, pancreas acute rejection rate was as low as 39%. Though indication for pancreas biopsy in these cases might have been because of hyperglycemia, the improved accuracy and, most importantly, the high sensitivity (85.7%) of dd-cfDNA along with the high PPV (85.7%) and NPV (93.7%) to diagnose P-BPAR could lead to a reduction in the number of required pancreas graft biopsies and aid clinicians in optimally timing indication biopsies.

The diagnosis of ABMR is an evolving field in solid organ transplantation. In pancreas transplantation, and according to the Banff classification,<sup>22</sup> the diagnosis of ABMR depends on the presence of characteristic histological lesions, presence of C4d staining, and circulating DSAs. The latter correlate not only with an increased risk for graft failure<sup>27,28</sup> but their presence has also been associated with subclinical acute rejection episodes. In a series of per protocol pancreas graft biopsies performed when DSA was identified, Uva et al<sup>29</sup> found that 46% of the patients presented had a subclinical pancreas graft rejection. Thus, interestingly, 54% of patients did not show signs of acute rejection despite having circulating DSAs. These results correlate with another recent study that explored gene expression during biopsy-proven ABMR in pancreas, which also found no correlation between the presence of DSA and ABMR gene expression.<sup>30</sup> While these studies highlight that though the presence of DSA increases the risk for acute rejection and consequent reduction in pancreas graft survival, they also remark that most of these patients do not develop ABMR. In our study, 3 patients who presented with DSAs in biopsies performed >45 d were observed to have higher median dd-cfDNA levels. Although 1 biopsy indicated ABMR, the other 2 showed no rejection and T cell-mediated rejection grade 3. Though these results cannot be extrapolated to other cohorts and the small sample size limits the conclusions that can be drawn, they pave the way toward larger and broader studies to evaluate the relevance of the concomitant use of DSA and dd-cfDNA to predict the diagnosis of ABMR in SPKTx recipients. The combination of these techniques could potentially reduce the requirement for protocol biopsies in cases in which de novo DSA is detected.

In the present study, biopsies with the Banff category “indeterminate for rejection” were classified as acute rejection and clinically treated accordingly. However, interpretation and treatment of indeterminate biopsies with mild histological features vary by physician,<sup>1,31</sup> and to date, no study has evaluated the long-term outcomes of different treatment

strategies on pancreas grafts with indeterminate biopsy results, which in some ways resemble the Banff borderline category in kidney transplantation.<sup>11</sup> When excluding indeterminate biopsies, dd-cfDNA levels were still significantly elevated compared with nonrejection cases. Despite the small size, these results pave the way toward the assessment of the value of dd-cfDNA, with or without DSA expression, to improve treatment strategies in SPKTx patients.

The authors would like to highlight some additional limitations to this study. First, this is a retrospective study where in only patients with matched pancreas biopsy-plasma samples were included. Second, the study was performed at a single center, and the decision for-cause pancreas biopsy was dependent on the attending physicians’ criteria; thus, extrapolation of these results to other centers must be done with care. Finally, all patients received a simultaneous kidney transplant from the same donor, but matched kidney biopsy data were not available for most samples in this study. Elevation of dd-cfDNA in kidney graft dysfunction is well described in literature and is the intended use of the Prospera test. Thus, it is not possible to exclude the possibility of confounding results because of dd-cfDNA released by the kidney graft, which could be experiencing rejection. We note that the absence of kidney biopsy data means that the performance data presented herein should be treated as a lower bound, as occult renal allograft rejection could explain some of the false-positive results. In a previous study,<sup>11</sup> 5 of 21 SPKTx patients (23%) showed biopsy-confirmed concomitant kidney and pancreas graft rejection. We also note that graft pancreatitis may also be associated with an increase in dd-cfDNA; however, we did not include any biopsies with acute pancreatitis and cannot draw any conclusions on how to interpret dd-cfDNA levels in such cases. Additionally, this study only included SPKTx patients and did not include any pancreas after kidney or pancreas transplant alone patients, and therefore, results shown herein cannot be extrapolated to these patients.

In conclusion, in this pilot study, we demonstrate for the first time that dd-cfDNA can discriminate between pancreas graft rejection and nonrejection in SPKTx recipients beyond day 45 after transplantation, with high sensitivity and specificity. These data may aid clinicians in the decision to perform or avoid a pancreas graft biopsy when graft dysfunction is suggested using the currently available clinical biomarkers.

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