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Subclinical Pancreas Rejection on Protocol Biopsy Within the First Year of Simultaneous Pancreas Kidney Transplant

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ABSTRACT

This single-center retrospective study investigated subclinical rejection prevalence and significance in simultaneous pancreas and kidney transplant (SPKT) recipients. We analyzed 352 SPKT recipients from July 2003 to April 2022. Our protocol included pancreas allograft surveillance biopsies at 1, 4, and 12months post-transplant. After excluding 153 patients unable to undergo pancreas biopsy, our study cohort comprised 199 recipients. Among the 199 patients with protocol pancreas biopsies, 107 had multiple protocol pancreas biopsies in the first year, totaling 323. Subclinical rejection was identified in 132 episodes (41%). Of these, 72% were Grade 1, 20% were indeterminate, and 8% were Banff Grade 2 or higher. All episodes of subclinical rejection were treated. Rates of pancreas graft loss (10% vs. 7%) and clinical rejection (21% vs. 20%) at 3 years were similar between those with and without subclinical rejection. Subclinical rejection Banff Grade 2 or more was associated with poor pancreas graft survival HR of 5.5 (95% CI: 1.24–24.37, $p = 0.025$). Of 236 simultaneous protocol kidney and pancreas biopsies, 102 (43%) showed pancreas subclinical rejection, while only 17% had concurrent kidney subclinical rejection. Our findings suggest limited predictive value of pancreatic enzymes and euglycemia in detecting pancreas rejection. Furthermore, poor concordance existed between pancreas and kidney subclinical rejection.

1 | Introduction

Simultaneous pancreas and kidney transplant (SPKT) confers a significant survival advantage, as it improves long-term metabolic and cardiovascular health [1]. More than 27 000 SPKT have been performed in the United States, with around 800–1000 patients receiving SPKT annually [2].

The role of rejection and chronic injury in pancreas graft loss remains unclear. Often, reported rejection rates are conservative, as many programs are reluctant to perform pancreas biopsies due to the risk of complications or limited resources. According to the Scientific Registry of Transplant Recipients (SRTR) report in 2016–2017, the incidence of acute pancreas rejection in the first year was 11.7%, 19.2%, and 12.4% for pancreas after kidney

Abbreviations: AR, acute rejection; BMI, body mass index; CMV, cytomegalovirus; DSA, donor specific antibody; HLA, human leukocyte antigen; KDPI, kidney donor profile index; PAK, pancreas after kidney; PTA, pancreas transplant alone; SPKT, simultaneous pancreas kidney transplantation; SRTR, Scientific Registry of Transplant Recipients; UMD, University of Maryland Classification System.

(PAK), pancreas transplant alone (PTA), and SPKT, respectively [2]. Rejection rates also vary based on the immunosuppression protocol adopted by different transplant centers [3–6]. Importantly, the diagnosis of pancreas rejection in SPKT cannot rely on kidney biopsy findings due to low concordance with pancreas rejection [7, 8].

Clinical pancreas rejection within 1 year has been associated with allograft loss and worse SPKT outcomes [4–6]. However, the incidence of subclinical rejection of pancreas biopsy in SPKT recipients is unavailable as surveillance biopsies are not performed. Consequently, the incidence and impact of subclinical rejection on the long-term pancreas graft function remain poorly documented. Subclinical rejection is defined as rejection identified on protocol pancreas biopsy without elevated pancreatic enzymes and/or hyperglycemia. In this study, we investigated the cross-sectional prevalence of subclinical rejection of the pancreas, its concordance with kidney allograft rejection, and its association with the incidence of future Clinical Rejection and pancreas allograft function.

2 | Methods

2.1 | Study Design and Population

The Mayo Clinic Institutional Review Board approved this study as a retrospective, single-center study of insulin-dependent diabetic patients receiving SPKT from July 2003 to April 2022. The last follow-up was at the end of September 2022.

Eligibility criteria for SPKT at our center included renal insufficiency combined with insulin-requiring diabetes or Pancreatic exocrine insufficiency. The following were exclusion criteria for SPKT: insulin requirement of >1 U/kg/day, body mass index (BMI) >35 kg/m^2 for patients with Type 1 diabetes, or BMI > 30 kg/m^2 for patients with Type 2 diabetes.

Standard surgical techniques for the pancreas allograft included using a donor iliac artery Y-graft for arterial reconstruction, systemic venous drainage into the recipient iliac vein or vena cava, and enteric drainage of the exocrine secretions.

All patients received induction immunosuppression. Before 2011, patients received induction with rabbit-anti-thymocyte globulin (r-ATG). After 2011, induction was changed to Alemtuzumab. Patients had complete withdrawal of corticosteroids by post-transplant Day 5. Steroids were maintained if they had panel reactive antibody (PRA) >80% or donor-specific antibody (DSA). Maintenance immunosuppression was tacrolimus and mycophenolate mofetil. Tacrolimus was started on post-transplant Day 1. Tacrolimus trough level goals were 8–10 ng/mL for the first month and 6–8 ng/mL afterward. We check mycophenolate trough levels at 1, 4, and 12 months.

We excluded patients with graft loss or death within the first 30 days, those with for-cause pancreas rejection within 30 days of transplant, and those recently treated for clinical rejection 45 days before the protocol biopsy.

2.2 | Outcomes

The primary endpoint was death-censored pancreas allograft loss, defined as needing >0.5 units/kg of insulin for at least 3 months.

We also examined the following:

1. Cross-sectional prevalence of subclinical rejection of the pancreas at 1 month, 4, and 12 months.
2. The rates of clinical rejection and pancreas allograft loss within the first 3 years.
3. Concordance of kidney rejection with pancreas rejection on protocol biopsy.
4. Hazard ratio of pancreas graft loss in those with no rejection, indeterminate, subclinical rejection excluding indeterminate and clinical rejection in first 3 years.
5. Death censored kidney graft survival and patient survival.

2.3 | Pancreas Allograft Biopsy

Our clinical protocol includes surveillance pancreas biopsies at 1 month (till 2018), 4, and 12 months. We also perform simultaneous kidney protocol biopsies. These were performed via ultrasound guidance with an 18-gauge automatic biopsy device. Our trajectory for biopsy was ideally toward the tail, avoiding the splenic artery and vein. If unsuccessful, or if there was no suitable safe window free of overlying bowel, then we proceeded with CT-guided biopsy using a posterior approach. Subclinical rejection was defined as rejection identified on protocol biopsy with normal pancreas enzyme levels and normoglycemia.

Banff's 2007 schema was utilized for histological interpretation. We perform C4D staining in all pancreas biopsies. The 2011 Banff update established comprehensive guidelines for diagnosing acute and chronic AMR. We monitor for DSA when graft dysfunction occurs and at 1, 4, and 12 months per protocol.

Before Banff grading, the University of Maryland Classification System (UMD) was used, and if a biopsy was graded using UMD, it was reclassified using Banff [10] (Supporting Information S1 describes The UMD and Banff description). Most grades of pancreas allograft rejection were treated with ATG, Grade 1 rejection episodes were treated with increased immunosuppression and steroids, while indeterminate were treated with increased immunosuppression ± steroids (Supporting Information S1 describes in detail the treatment regimen for subclinical rejection for pancreas transplant). Patients with Banff Grade 1 or higher rejection were maintained on prednisone 5 mg daily. The tacrolimus goal was increased to 8–10 ng/mL for 1 month after rejection and then maintained on dose targeting trough levels of 6–8 ng/mL. Mycophenolate dose was increased to 1 g twice daily if tolerated with a minimal goal between 2 and 4 ng/mL (Supporting Information S1). If patients are on reduced mycophenolate of 500 mg or less per day, prednisone is added per our protocol. For infection prevention after treatment of rejection with steroids or depleting agents, patients received Bactrim and Fluconazole for 3 months. In CMV mismatch recipients who received intravenous steroids, valganciclovir prophylaxis was given for 4 weeks.

For infection prophylaxis after lymphocyte-depleting therapy in CMV D+ or R+, we use valganciclovir prophylaxis for 12 weeks and in CMV D−/R− Acyclovir for 4 weeks.

Pancreas failure was defined as requiring >0.5 units/kg of insulin for at least 3 months. We also examined how many patients required >0.1 units/kg of insulin for at least 3 months. We included an insulin requirement of 0.1 units/kg for 3 or more months to help question some insulin requirements due to chronic rejection or high-dose steroids.

2.4 | Statistical Analysis

We compared the baseline characteristics and outcomes between the groups. Descriptive statistics were reported as mean (standard deviation) for continuous variables and frequency (percentage) and median and interquartile range for categorical variables. T-test was used for continuous variables comparing two groups, one-way variance analysis was used for three groups, and chi-square was used for dichotomous variables. Nonparametric tests compared data that were heavily skewed.

Kaplan–Meier survival analysis was done for death-censored pancreas, kidney, and patient survival. We used 365 days as the starting point and excluded those <365 days of follow-up/death or pancreas loss for pancreas survival. Patients with subclinical rejection in 1 year and no subclinical rejection in 1 year were analyzed.

As the impact of indeterminate changes is poorly understood, we also analyzed the survival of pancreas grafts, death-censored after excluding indeterminate rejection episodes. Additionally, we examined death-censored pancreas survival based on the number of subclinical rejection episodes, excluding indeterminate subclinical rejection.

We also compared the risk of pancreatic graft loss in group with no rejection, indeterminate rejection, subclinical rejection excluding the indeterminate and history of clinical rejection in the first 3 years of transplant using Cox proportional hazard analysis. Cox-proportional hazard regression with time-varying covariate analysis was done to evaluate the impact of the subclinical rejection grade on death-censored pancreas graft survival. The event was defined as the occurrence of the first subclinical rejection episode. If a patient had subclinical rejection at 1 month, this was considered the event time, regardless of any subsequent rejection episodes.

We used SPSS (version 28, IBM, Armonk, NY) for analysis.

3 | Results

3.1 | Study Population

A total of 352 patients received SPKT from July 2003 to April 2022. Within 30 days of the transplant, 34 patients experienced pancreas graft loss ($n = 33$, 9%) or death ($n = 1$). Figure 1 shows the study flow chart. One hundred ninety-nine had a protocol biopsy of pancreas graft within 1 year.

Of the 199 patient cohorts who had protocol biopsy, 54.3% (108) patients had the presence of subclinical rejection within the first year. Of the 199 patients who underwent protocol pancreas biopsy, 107 had more than one protocol biopsy in the first year.

Of 323 protocol biopsies performed, 132 (41%) episodes of subclinical rejection in the pancreas were identified.

At 1 month, subclinical rejection of the pancreas was observed in 32% (29/92) of biopsies, at 4 months in 46% (62/135) of biopsies, and 12 months in 43% (41/96) of biopsies (Figure 2).

Across the 132 episodes of rejection, 72% (95/132) were classified as Grade 1, 20% (26/132) were indeterminate, and 8% (11/132) were Banff Grade 2 or higher. Among these, we had two cases of C4d positive rejection but were in the absence of donor-specific antibodies, and the diagnosis of antibody-mediated rejection was not met based on the Banff criteria.

3.2 | Concordance of Subclinical Pancreas and Kidney Rejection When Performed Simultaneously (Figure 3a–c)

Additionally, we studied the concordance of kidney and pancreas subclinical rejection on protocol biopsies. There were 236 simultaneous pancreas and kidney protocol biopsies performed.

Simultaneous protocol biopsies of the pancreas and kidney were carried out for 236 patients, of which 102 revealed subclinical rejection of the pancreas. Among these 102 patients, only 17% (17/102) also had kidney subclinical rejection. Figure 3a–c describes the results of the simultaneous biopsies with rejection grades.

At 1 month, 19 had rejection of one or more organs. Eighteen patients had pancreas rejection, and only three had simultaneous kidney rejection.

Concordance for no rejection of both organs at 1 month was 59% (27/46), 53% (55/104) at 4 months, and 53% (46/86) at 12 months.

Concordance for rejection in both organs at 1 month was 6% (3/46), 12% (13/104) at 4 months, and 1% (1/86) at 12 months.

Episodes of kidney rejection without pancreas rejection at 1 month were 2% (1/46), 0% at 4 months, and 6% (5/86) at 12 months.

Episodes of pancreas rejection with the absence of kidney rejection at 1 month were 33% (15/46), 35% (36/104) at 4 months, and 40% (34/86) at 12 months.

3.3 | Characteristics of the Group With Subclinical Rejection vs. No Subclinical Rejection on the Pancreas Protocol Biopsy (Tables 1 and 2)

One hundred ninety-nine patients had protocol biopsy, 91 patients had no subclinical rejection on protocol biopsy, while 108 patients had subclinical rejection on protocol biopsy within the first year. Both groups had an average age of 45 years. Females represented

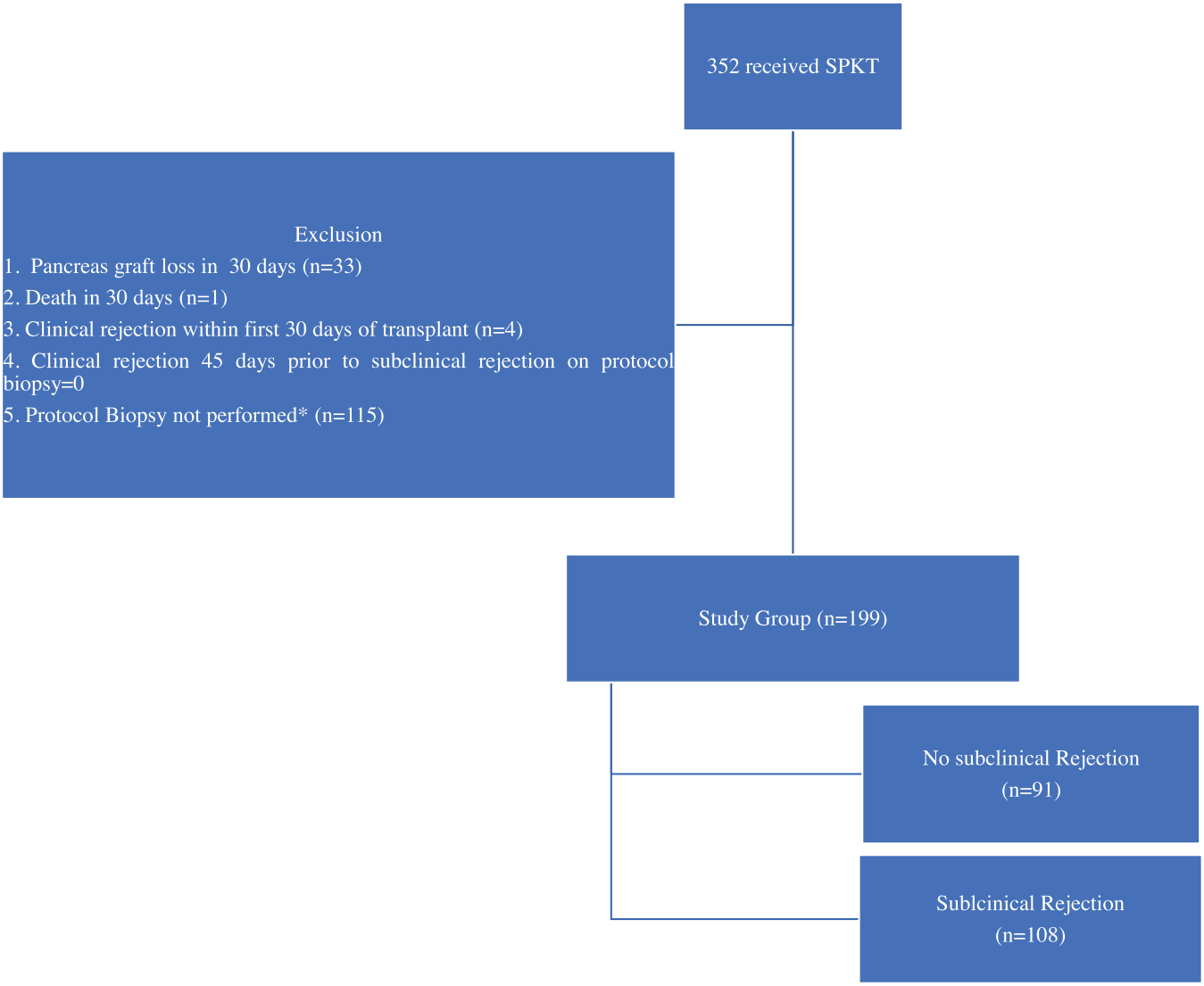


FIGURE 1 | Study flow chart. *Reasons for not performing protocol pancreas biopsy were anticoagulated (17), overlying bowel gas (16), Jehovah's Witness (2), history of pancreatitis (9), declined by patient (9), hypotension (1), hematoma (1), pancreas not visualized (5), hernia mesh interfering with doing a pancreas biopsy (1), due to early posttransplant issues (leak) (2), peripancreatic abscess (2), history of gastrointestinal bleed (1), fluid collection (1), and not known (48).

43% of the subclinical rejection group, compared to 39% without subclinical rejection (Table 1). Both groups had similar recipient and donor characteristics.

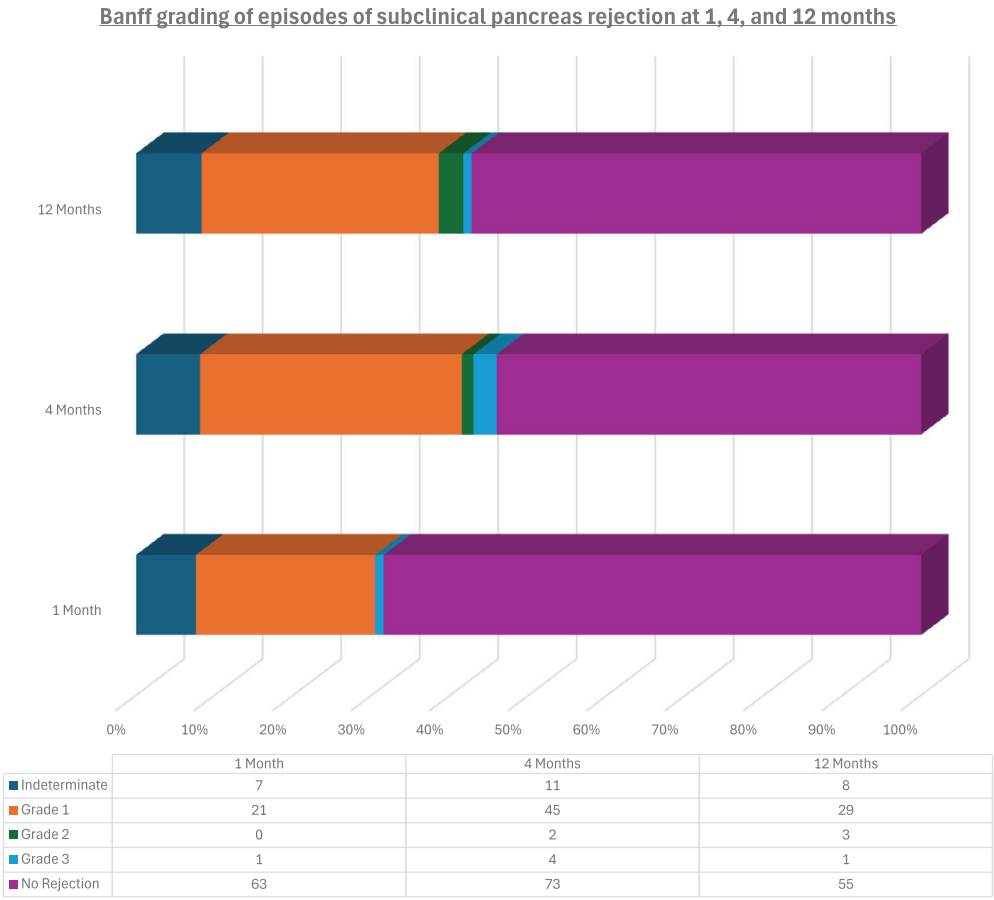
The incidence of cytomegalovirus (CMV) and BKV was not different between the groups. In the subclinical rejection group, 12 patients had CMV viremia 3 months prior to the protocol biopsy, and 12 patients had BKV Viremia, warranting a reduction in their immunosuppression due to viremia. Five patients developed CMV viremia, and two developed BKV viremia within 3 months of subclinical rejection treatment (Table 1).

We analyzed the immunosuppressant levels at the time of protocol biopsy and compared the group with subclinical rejection episodes with those without subclinical rejection. At 1 month, most of the patients were within the target range. At 4 months, more patients with subclinical rejection had FK levels <6 ng/mL than those without subclinical rejection (34% vs. 18%, $p = 0.003$) (Table 2). At 4 months, there was a trend for lower mycophenolate levels in those with subclinical rejection and without subclinical rejection. At 12 months, mycophenolate level was lower in those with versus without subclinical rejection, 3 (1.6) versus 4 (2) mcg/mL, $p = 0.03$.

Serum lipase, Hba1c, and C-peptide were similar in those with or without subclinical rejection at 1, 4, and 12 months.

3.4 | Pancreas, Kidney, and Patient Outcomes of Groups With Treated Subclinical Rejection vs. No Subclinical Rejection on the Pancreas Protocol Biopsy (Tables 3 and 4)

In the group with treated subclinical rejection versus no subclinical rejection, the primary outcome of pancreas graft loss was similar: 10% (11/108) graft loss in the group with subclinical



	1 Month	4 Months	12 Months
Indeterminate	7	11	8
Grade 1	21	45	29
Grade 2	0	2	3
Grade 3	1	4	1
No Rejection	63	73	55

FIGURE 2 | Banff grading of episodes of subclinical pancreas rejection at 1, 4, and 12 months.

rejection and 7% (6/91) in those without subclinical rejection up until the last follow-up (Tables 3 and 4). The rate of pancreas graft loss within 3 years or the need for insulin for more than 3 months in the same period was comparable between the two groups (Table 4). Specifically, 4.6% (5 out of 108) in the group with subclinical rejection and 4.4% (4 out of 91) without subclinical rejection experienced pancreas graft loss within 3 years.

In the group with treated subclinical rejection, the 3-year incidence of clinical rejection of the pancreas was 21%, similar to the 20% observed in those without subclinical rejection. The group that did not undergo a protocol biopsy had a 1-year incidence of clinical rejection at 23%, increasing to 28% at 3 years.

A significant number of patients were on steroids by 12 months; 80% of patients who experienced subclinical rejection were on steroids by then, compared to only 20% of those without subclinical rejection.

For patients with treated subclinical rejection versus those without subclinical rejection, the incidence of pancreas graft loss or clinical rejection within 3 years was likewise similar, standing at 25% (27 out of 108) in the subclinical rejection group and 23% (21 out of 91) in the non-subclinical rejection group ($p = 0.87$).

In the group with treated subclinical rejection versus no subclinical rejection, the formation of de novo DSA within 1 year was not significantly different, with a rate of 16% (17/108) versus 10% (7/91) ($p = 0.4$), respectively. However, kidney rejection, including subclinical rejection, was significantly higher in the group with subclinical pancreas rejection, with a rate of 26% (28/108) compared to 10% (9/91) in those without subclinical rejection of the pancreas ($p = 0.006$).

3.4.1 | Survival Analysis

Kaplan–Meier survival analysis showed that the death-censored pancreas graft survival was similar between the two groups with and without subclinical rejection at 1 year (Log-rank p value = 0.9) (Figure 4a). After excluding indeterminate rejection episodes from the subclinical rejection group, the death-censored survival of pancreas grafts remained similar between the groups with and without subclinical rejection ($p = 0.253$). We also analyzed death-censored pancreas survival based on the number of episodes of subclinical rejection, excluding indeterminate cases. Patients with two or more episodes of subclinical rejection had poorer graft survival than those with none or one episode of subclinical rejection ($p = 0.05$) (Figure 4b).

On cox-proportional hazard regression with time-varying covariate analysis, subclinical rejection Banff Grade 2 or more was associated with poor pancreas graft survival HR of 5.5 (95% CI 1.24–24.37, $p = 0.025$).

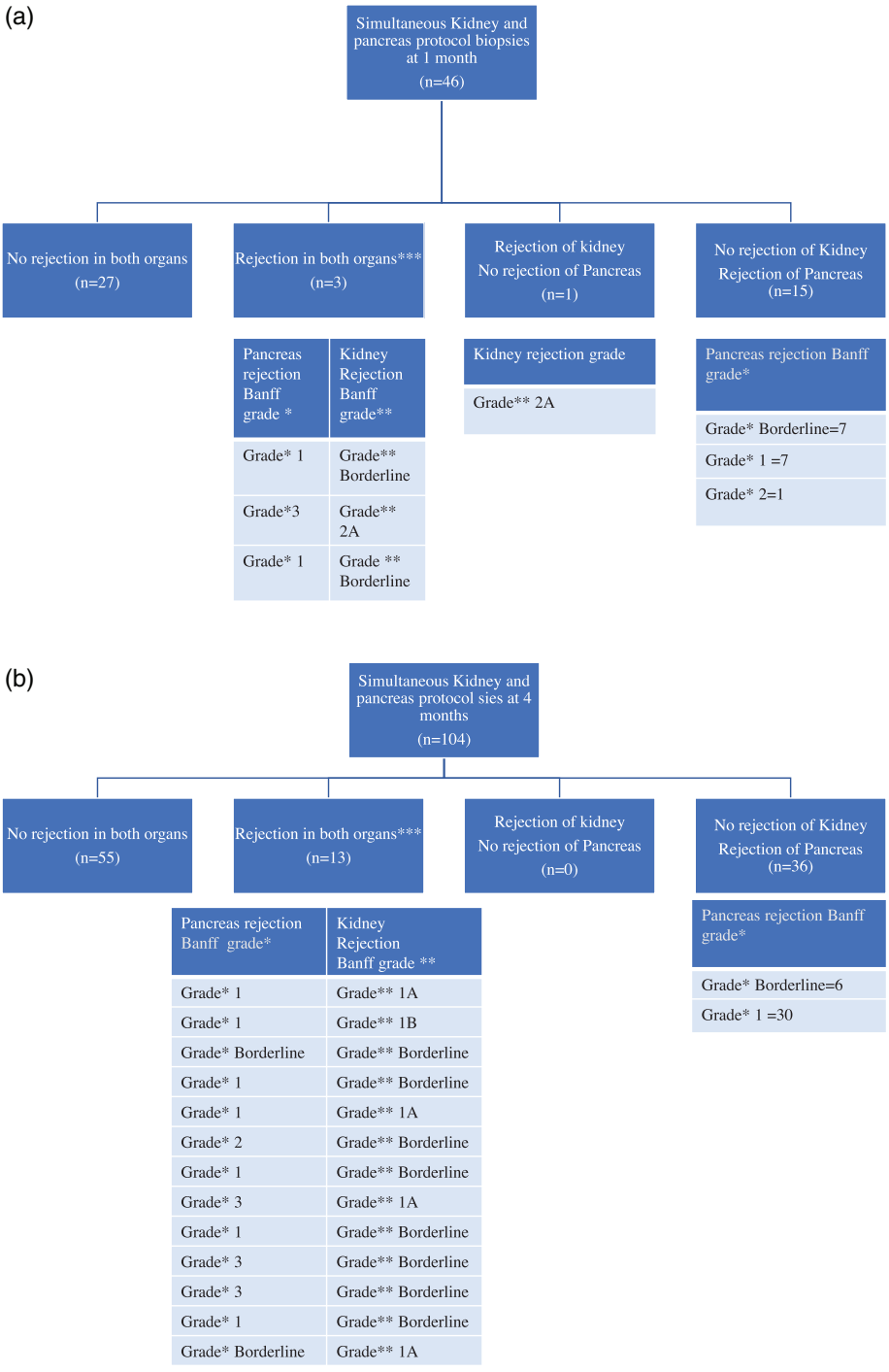


FIGURE 3 | (a)–(c) Concordance of subclinical pancreas and kidney rejection. (a) Concordance of subclinical pancreas and kidney rejection when performed simultaneously at 1 month. (b) Concordance of subclinical pancreas and kidney rejection when performed simultaneously at 4 months. (c) Concordance of subclinical pancreas and kidney rejection when performed simultaneously at 12 months. *Pancreas Rejection Banff grading. **Kidney rejection Banff grading. ***When there was rejection noted in both organs, the figure shows concomitant histological biopsy grading in the pancreas and kidney for the patient.

Clinical rejection was associated with a significantly increased risk of pancreas failure, with a hazard ratio (HR) of 4.557 [95% CI: 1.682, 12.348, $p = 0.003$]. Subclinical rejection showed a trend toward increased risk of pancreas failure, with an HR of 2.525 [95% CI: 0.935, 6.817, $p = 0.068$], but this result is not statistically significant. Indeterminate cases of subclinical rejection do not show an increase in the risk of pancreas failure, with an HR of 2.070 [95% CI: 0.428, 10.006, $p = 0.366$] when compared to the group with no rejection

Death-censored kidney graft survival was similar between groups with or without subclinical rejection (Log-rank p value = 0.7). Patient survival was also similar between those with subclinical or no subclinical rejection (Log-rank p value = 0.9).

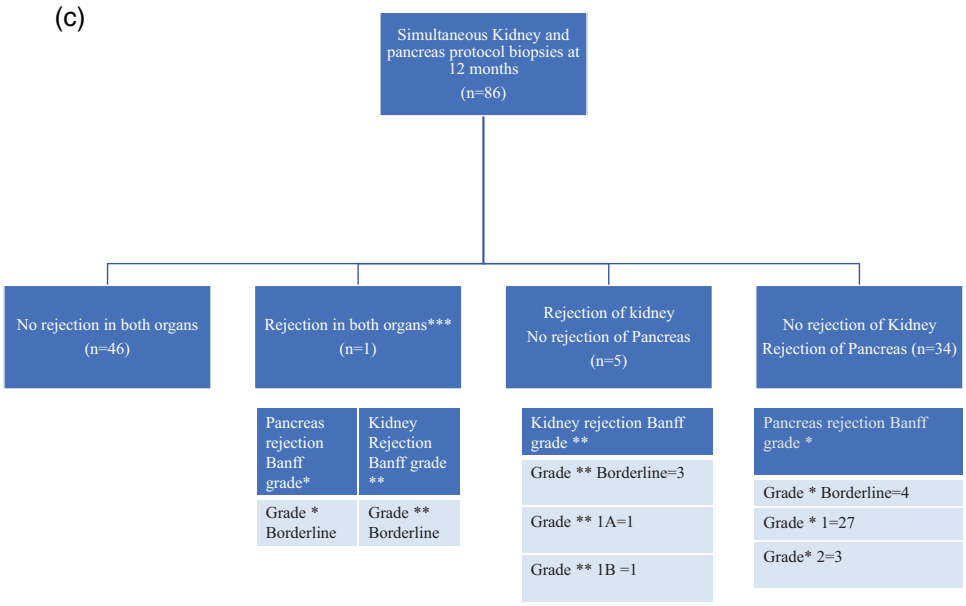


FIGURE 3 | (Continued)

TABLE 1 | Subclinical rejection vs. no subclinical rejection on the pancreas protocol biopsy.

	No subclinical rejection of pancreas on protocol biopsy within the first year ($n = 91$)	**Subclinical rejection of pancreas on protocol biopsy within the first year ($N = 108$)**	***p* value**
Age	45.5 (9.8)	45 (10.4)	0.79
Race (White/Black/Hispanic/others)	46%/8%/31%/15%	64%/5%/25%/5%	0.1
Sex (female)	35 (39%)	46 (43%)	NS
Body mass index (kg/m^2)	25.4 (3.6)	25.5 (3.7)	0.87
Type 1 diabetes mellitus	67 (74%)	83 (77%)	0.6
Previous kidney transplant	5 (5.5%)	4 (4%)	0.5
Previous Pancreas transplant	4 (4%)	2 (2%)	0.3
HLA mismatch	4.7 (1.2)	4.4 (1.2)	0.36
Kidney donor profile index	14.8 (12)	15.6 (14)	0.72
Creatinine at discharge (mg/dL)	1.8 (1.5)	1.7 (1)	0.66
Cold ischemia time kidney (h)	7.3 (3.3)	6.8 (3.2)	0.54
Median years of follow-up (IQR)	5.8 (3.5–11)	7 (4.2–11.2)	
BKV Viremia	20 (22%)	23 (21%)	1
CMV Viremia	11 (12%)	24 (22%)	0.06
CMV Viremia 3 months before the subclinical rejection		12 (11%)	
CMV Viremia 3 months after the subclinical rejection		5 (4.6%)	
BKV Viremia 3 months before subclinical rejection		12 (11%)	
BKV Viremia 3 months after the episode of subclinical rejection		2 (1.8%)	

TABLE 2 | Immunosuppressant levels at 1, 4, and 12 months.

Immunosuppressant levels at 1 month			
	No subclinical rejection at 1 month (63)	**Subclinical rejection at 1 month (29)**	***p* value**
Tacrolimus level <8 ng/mL			**0.2**
[a]Missing	[a]0	[a]0	
Yes	22 (35%)	7 (22%)	
No	41 (65%)	22 (78%)	
Mycophenolate level mcg/mL, mean (Standard deviation)	3.1 (2)	3.5 (1.6)	**0.4**
Immunosuppressant levels at 4 months			
	No subclinical rejection at 4 months (73)	**Subclinical rejection at 4 months ($n = 62$)**	***p* value**
Tacrolimus level <6 ng/mL			0.03
[a]Missing	0	0	
Yes	18% (13/73)	34% (21/62)	
No	82% (60/73)	66% (41/62)	
Mycophenolate level mcg/mL, mean (standard deviation)	3.9 (2.7)	3 (1.9)	0.06
Immunosuppressant levels at 12 months			
	No subclinical rejection at 12 months (55)	**Subclinical rejection at 12 months (41)**	
Tacrolimus level <6 ng/mL			0.34
[a]Missing	[a]3.5% (2/55)	[a]7.5% (3/41)	
Yes	22% (12/55)	29.5% (12/41)	
No	74.5% (41/55)	63% (26/41)	
Mycophenolate level mcg/mL, mean (standard deviation)	4 (2)	3 (1.6)	0.03

[a]Missing variables were excluded in the Chi-square analysis.

3.4.2 | Complications of Protocol Biopsy

We were able to review the complications of biopsies since 2015; there were 162 protocol biopsies during that time. Among these 162 protocol biopsies, 6 (3.7%) complications were noted. The complications included 3 (1.85%) patients with bleeding; none of them required transfusion. Three patients (1.85%) had an elevation in amylase and lipase, which were attributed to biopsy, as were normal in the morning before the biopsy. Of these six patients, three were monitored overnight in the hospital.

4 | Discussion

Since most pancreas allografts are not routinely biopsied, the prevalence and outcomes of subclinical rejection remain poorly understood. Our study found a high prevalence of subclinical rejection within the first year post-SPKT, identifying 132 episodes (40.8%) among 323 protocol biopsies, with 55% of biopsied patients exhibiting subclinical rejection. These findings highlight the limited sensitivity of serum pancreas enzymes and hyperglycemia in predicting rejection.

Furthermore, the high rates of subclinical rejection and a significant number of patients resuming steroids raise questions about the safety and efficacy of early steroid elimination as a maintenance immunosuppressive regimen in this patient population.

Our observations show a significant discordance between pancreas and kidney subclinical rejection, indicating that kidney biopsies cannot reliably act as surrogates for pancreas rejection. Studies have shown that rejection in both organs can occur independently, even when dysfunction exists in one or both [8]. In a study of 101 concurrent biopsies from 70 patients with organ dysfunction, only 40% showed concurrent rejection, with 33.5%

TABLE 3 | Outcomes of groups with subclinical rejection versus no subclinical rejection on the pancreas protocol biopsy.

	No subclinical rejection on protocol biopsy within the first year ($n = 91$)	Subclinical rejection on protocol biopsy within the first year ($N = 108$)	p value
Pancreas graft loss or clinical rejection in 3 years	21 (23%)	26 (25%)	0.87
Pancreas clinical rejection rate within first 3 years Grades: Indeterminate/1/2/3	21 (23%) 1%/10%/9%/3%	25 (23%) 3%/10%/4.6%/5.6%	0.63
Pancreas clinical rejection rate after excluding clinical rejection before subclinical rejection	20 (21%)	21 (19%)	0.8
Kidney rejection, including subclinical rejection in the first 1 year	9 (10%)	28 (26%)	0.006
Pancreas graft loss within 3 years (>0.5 units/kg for >90 days)	4 (4%)	5 (5%)	1
Insulin requirement >0.1 units/kg for >90 days within 3 years	4 (4%)	5 (5%)	1
De Novo donor-specific antibody within 1 year	7 (10%)	17 (16%)	0.4

TABLE 4 | Causes of pancreas graft loss.

	No subclinical rejection on protocol biopsy ($n = 91$)	Subclinical rejection on protocol biopsy within the first year ($N = 108$)
Graft loss	6 (6.5%)	11 (10%)
Acute rejection	0	1
Chronic rejection	3	7
Discontinuation of immunosuppressants	2	0
Pancreatitis	0	1
Insulin resistance	0	1
Leak	1	0
Peri pancreatic infection	0	1

showing kidney-only rejection and 26.5% showing pancreas-only rejection. Our findings support this lack of concordance, with pancreas rejection grades often higher. In over half of concurrent rejection cases, the pancreas more often demonstrated a higher rejection grade [7]. This lack of concordance, often with higher grades in pancreas rejections, underlines the necessity for specific pancreas biopsies when clinically indicated.

In our study, the incidence of indeterminate changes was 8% at 1, 4, and 12 months. Without active treatment, the outcomes of these mild subclinical rejections are uncertain. For instance, a previous study on 15 patients with minimal (Drachenberg Grade II) rejection on surveillance biopsy found that without specific treatment like steroids or Thymoglobulin, 40% improved, but 60% remained the same or worsened, and some progressed to more severe grades [11]. It is worth noting that these transplant recipients were managed with higher immunosuppression levels than current practices. These findings raise uncertainty regarding whether indeterminate pancreas subclinical rejection or even Banff Grade 1 rejection, would resolve or progress without subclinical rejection treatment under our current immunosuppression regimen.

Patients with subclinical rejection were on lower immunosuppression, and a significant number had CMV or BKV viremia. This observation suggests the need for vigilant monitoring and optimized baseline immunosuppression in these patients. Furthermore, the group with subclinical was at higher immunological risk, as suggested by the higher subclinical and clinical rejection of the kidney and higher DSA in the group with pancreas subclinical rejection than those without subclinical rejection. Patients with two or more episodes of subclinical rejection had poor graft survival compared to none or one episode of subclinical rejection. This may indicate that the group with subclinical rejection of the pancreas may have a primed immune system and, therefore, is at higher risk.

As the group with subclinical rejection was at an immunologically higher risk than those without subclinical rejection and were treated, it is unclear if the similar survival is due to treatment. There is limited data on the role of surveillance pancreas graft biopsy and its treatment.

A previous study has highlighted a decreased probability of graft survival, as observed through the identification of pancreas rejection on surveillance biopsy in recipients of PAK and PTA [5]. Rejection identified through protocol biopsy had an increased risk of complete graft failure (HR 4.62, 95% CI 1.79–

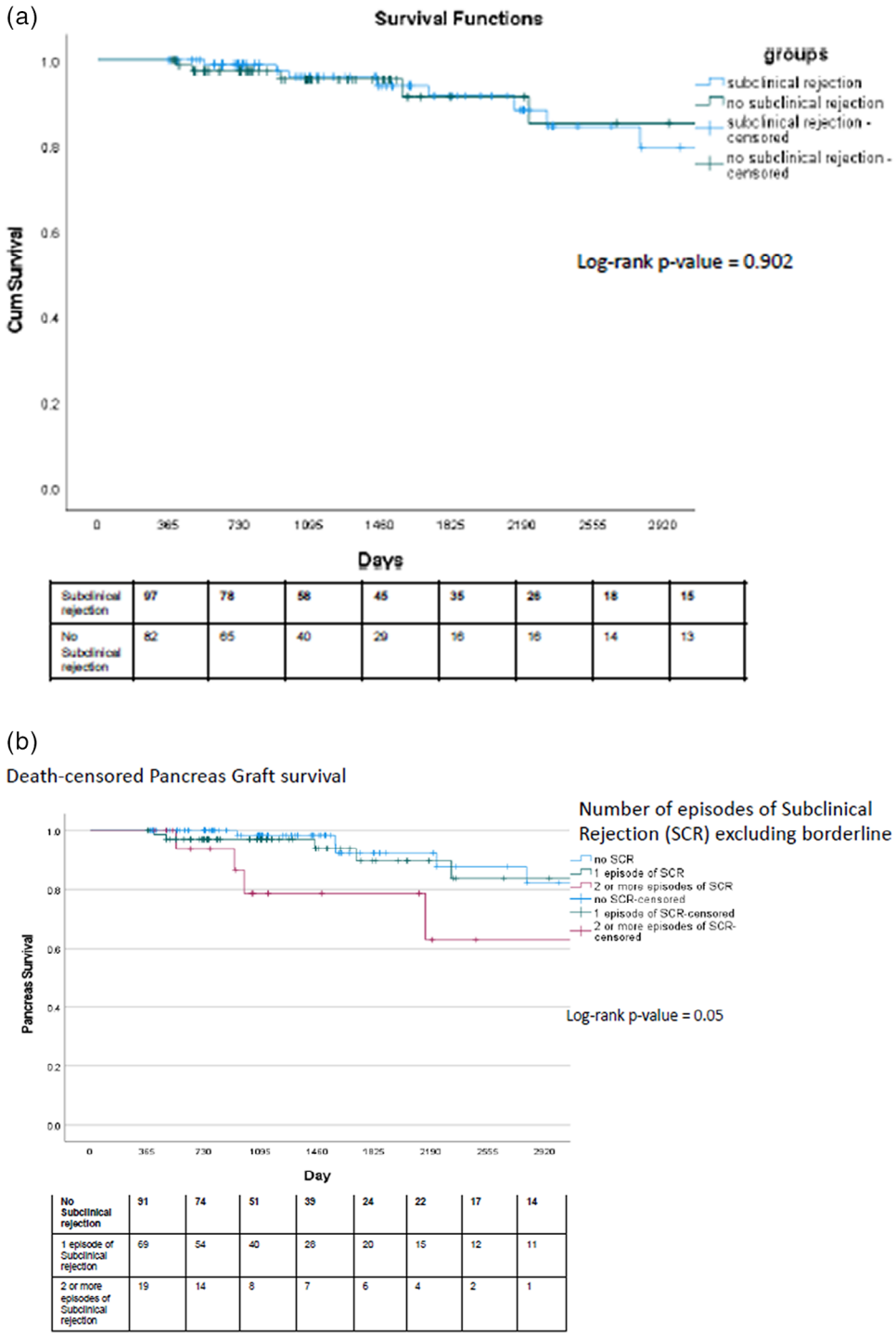


FIGURE 4 | (a) and (b) Kaplan–Meier survival analysis. (a) Kaplan–Meier survival analysis death-censored pancreas graft survival in patients with and without subclinical rejection. (b) Kaplan–Meier survival analysis death-censored pancreas graft survival in patients depending on the episodes of Subclinical rejection in the first year.

11.9) and a marginally significantly increased risk of partial failure (HR 2.17, 95% CI 0.97–4.85) compared to those with clinical rejection [5].

Casey et al. reported that severe acinar inflammation, acinar fibrosis, and vascular luminal narrowing from chronic rejection correlated significantly with poor outcomes, and milder inflammation did not appear to impact graft outcomes [11]. In our study, higher rejection grades, such as Banff Grade 2 or more, were associated with the risk of pancreas graft loss.

The incidence of clinical rejection and pancreas graft loss was comparable in the groups with subclinical rejection versus no subclinical rejection. Given that subjects with subclinical rejection were treated, similar graft outcomes may be a consequence of the treatment. It remains unclear whether detection and

treatment of subclinical rejection provide an added value in preventing graft loss. This practice of conducting surveillance biopsies comes with the disadvantage of incurring costs and the potential for complications associated with protocol biopsies, which may lead to reconsidering its value relative to its benefits. Additionally, treatment of subclinical rejection also subjects patients to significantly increased immunosuppression, which may be associated with their complications.

Notably, the group with subclinical rejection was on lower levels of immunosuppression, had a higher incidence of de novo DSA at year 1, and experienced higher kidney rejection rates compared to those without subclinical rejection. This may indicate that the group with subclinical pancreas rejection might be at higher immunological risk, where early detection could have prevented clinical rejection. However, patients who are under-immunosuppressed are more likely to be identified as having subclinical rejection, suggesting that efforts should be devoted to optimizing baseline immunosuppression.

Since all episodes of subclinical rejection were treated, it is unclear of the natural course as to whether it would have progressed to rejection, resolved, or was an artifact of interpretation [11]. Our study, like many in the field, treated all identified episodes of subclinical rejection, guided by prevailing clinical practices and the ethical obligation to minimize the risk of graft loss. However, this approach complicates the ability to delineate the natural progression of these subclinical findings. As noted in the article by Casey et al. [11] there are historical precedents where untreated minimal subclinical rejection did not uniformly progress to higher rejection grades, suggesting a potential for spontaneous resolution or stable indolence. However, the comparison to current practices is limited, as these studies were conducted under different immunosuppressive regimes and clinical protocols.

Furthermore, the treatment of all identified episodes of subclinical rejection in our study was consistent with the imperative to minimize the risk for our patient population, given the significant consequences of graft loss. This approach aligns with our findings that subclinical rejection, especially when Banff Grade 2 or higher, was associated with worse graft survival outcomes (HR of 5.5, 95% CI: 1.24–24.37, $p = 0.025$).

It is also crucial to acknowledge the gaps in our understanding due to not performing immunophenotyping of the infiltrating cells during these subclinical rejection episodes. Our study did not include staining for subtypes of T lymphocytes or cytokines, which might have provided insights into the nature of the immune response and its role in tolerance induction or rejection progression. This limitation leaves significant unanswered questions about the specific characteristics of the infiltrate observed and its implications for the allograft's fate.

The First World Consensus Conference on Pancreas Transplantation highlighted that there is no conclusive evidence supporting the routine use of protocol biopsies in SPKT recipients [12]. The implementation of these biopsies, whether for the pancreas or kidney, remains largely center-specific and is typically part of immunologic surveillance. The consensus panel acknowledged the variability in clinical practice and the absence of standardized guidelines, reflecting the ongoing uncertainties surrounding the role of surveillance biopsies. Our study, which involved protocol pancreas biopsies at 1, 4, and 12 months posttransplant, revealed significant discordance between pancreatic and renal biopsy results. This finding aligns with the consensus panel's observation that kidney biopsies alone cannot reliably determine pancreas rejection status, emphasizing the need for direct pancreas surveillance in SPKT recipients. These results support the necessity for more prospective studies to clarify the role of protocol biopsies in improving patient outcomes in SPK transplantation.

This study has several limitations. It is a retrospective, observational cohort study and is restricted by the number and geographic location of the study population, being a single-center study. Subclinical rejection was treated, making it unclear if similar survival in the groups with and without subclinical rejection was a result of the treatment. We did not perform staining for subtypes of T lymphocyte cells and cytokines, leaving unanswered questions about the specific infiltrate observed in the allograft during subclinical T-cell mediated rejection episodes and its potential role in tolerance induction. We did not perform a follow-up biopsy at 4–6 weeks to determine the histological resolution of rejection.

Furthermore, we use early steroid withdrawal; it is unclear if early steroid withdrawal could have contributed to the high rates of subclinical rejection. Biomarkers like donor-derived cell-free DNA and gene expression profiles lack validation and approval for use in SPKT [13]. Future research should explore their correlation with subclinical rejection and prognosis. These biomarkers may also be valuable for monitoring patients with reduced immunosuppression, particularly those with viral infections or others at rejection risk.

It is important to note that acute rejection is not a homogenous molecular entity and comprehensive studies involving microarray analysis and genetic alterations in a subclinical rejection are necessary for a deeper understanding. Normal serum pancreas enzyme levels, normoglycemia, and kidney biopsies have shown poor predictive value for subclinical pancreas rejection in SPKT.

5 | Conclusion

This study demonstrates that subclinical rejection is prevalent in the first year post-SPKT and highlights the limited predictive value of serum pancreas enzymes and normoglycemia for rejection. It remains unclear whether early steroid withdrawal contributed to the high rates of subclinical rejection. The discordance between subclinical pancreas and simultaneous kidney rejection suggests that kidney biopsies should not be used as surrogates for pancreas rejection. Patients with subclinical rejection often had lower levels of immunosuppression and higher immunological risk, indicating the potential benefit of early detection and treatment. Despite similar graft survival rates between patients with and without subclinical rejection, optimizing baseline immunosuppression may be necessary to address the higher risk associated with subclinical rejection. Future research

should investigate the role of emerging biomarkers in predicting and managing subclinical rejection.

Author Contributions

P.B., R.L.H., K.S.R., and H.A.C. conceived the idea. All the participants participated in the writing of the paper and the performance of the research.

Disclosure

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

1. C. Morath, M. Zeier, B. Dohler, J. Schmidt, P. P. Nawroth, and G. Opelz, "Metabolic Control Improves Long-Term Renal Allograft and Patient Survival in Type 1 Diabetes," *Journal of the American Society of Nephrology* 19, no. 8 (2008): 1557–1563.

2. R. Kandaswamy, P. G. Stock, J. Miller, et al., "OPTN/SRTR 2020 Annual Data Report: Pancreas," *American Journal of Transplantation* 22 (2022): 137–203.

3. S. A. White, J. A. Shaw, and D. E. Sutherland, "Pancreas Transplantation," *Lancet* 373, no. 9677 (2009): 1808–1817.

4. A. Humar, K. Khwaja, T. Ramcharan, et al., "Chronic Rejection: The Next Major Challenge for Pancreas Transplant Recipients," *Transplantation* 76, no. 6 (2003): 918–923.

5. M. Dong, A. K. Parsaik, W. Kremers, et al., "Acute Pancreas Allograft Rejection is Associated With Increased Risk of Graft Failure in Pancreas Transplantation," *American Journal of Transplantation* 13, no. 4 (2013): 1019–1025.

6. S. Yamamoto, G. Tufveson, J. Wahlberg, C. Berne, J. Wadstrom, and A. R. Biglarnia, "Factors Influencing Outcome of Simultaneous Kidney and Pancreas Transplantation: A 23-Year Single-Center Clinical Experience," *Transplantation Proceedings* 42, no. 10 (2010): 4197–4201.

7. P. D. Uva, J. C. Papadimitriou, C. B. Drachenberg, et al., "Graft Dysfunction in Simultaneous Pancreas Kidney Transplantation (SPK): Results of Concurrent Kidney and Pancreas Allograft Biopsies," *American Journal of Transplantation* 19, no. 2 (2019): 466–474.

8. S. Parajuli, E. Arpali, B. C. Astor, et al., "Concurrent Biopsies of Both Grafts in Recipients of Simultaneous Pancreas and Kidney Demonstrate High Rates of Discordance for Rejection as Well as Discordance in Type of Rejection—A Retrospective Study," *Transplant International* 31, no. 1 (2018): 32–37.

9. J. C. Papadimitriou, C. B. Drachenberg, A. Wiland, et al., "Histologic Grading of Acute Allograft Rejection in Pancreas Needle Biopsy: Correlation to Serum Enzymes, Glycemia, and Response to Immunosuppressive Treatment," *Transplantation* 66, no. 12 (1998): 1741–1745.

10. C. B. Drachenberg, J. Odorico, A. J. Demetris, et al., "Banff Schema for Grading Pancreas Allograft Rejection: Working Proposal by a Multi-Disciplinary International Consensus Panel," *American Journal of Transplantation* 8, no. 6 (2008): 1237–1249.

11. E. T. Casey, T. C. Smyrk, L. J. Burgart, M. D. Stegall, and T. S. Larson, "Outcome of Untreated Grade II Rejection on Solitary Pancreas Allograft Biopsy Specimens," *Transplantation* 79, no. 12 (2005): 1717–1722.

12. U. Boggi, F. Vistoli, A. Andres, et al., "First World Consensus Conference on Pancreas Transplantation: Part II—Recommendations," *American Journal of Transplantation* 21, no. 3 (2021): 17–59.

13. P. Ventura-Aguiar, M. J. Ramirez-Bajo, J. Rovira, et al., "Donor-Derived Cell-Free DNA Shows High Sensitivity for the Diagnosis of Pancreas Graft Rejection in Simultaneous Pancreas-Kidney Transplantation," *Transplantation* 106, no. 8 (2022): 1690–1697.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.