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Research Article

Outcomes Of Pancreatic Biopsies In Simultaneous Pancreas And Kidney (Spk) Transplantation For Suspected Acute Rejection:.

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INTRODUCTION

SPK is currently the optimal therapeutic approach to enhance both quality of life and overall survival in highly selected patients with type 1 or type 2 diabetes and end-stage renal disease[1-3].

The first successful pancreas transplantation was performed in 19664,5. By 2014, over 48,000 pancreas transplants had been performed worldwide, with numbers continuing to rise[5,6].

During the post-transplant follow-up of SPK, close monitoring is essential, encompassing clinical evaluation, laboratory parameters and imaging studies. Although multiple biological biomarkers are useful in detecting acute rejection, amylase and lipase levels remain the parameters across centers due to their sensitivity exceeding 70%.

In fact, Serum amylase and lipase levels are routinely monitored postoperatively and should remain within normal ranges. However, elevations in these enzyme levels, although very useful, are relatively common, often complicating the differential diagnosis. In such cases, pancreatic biopsy is frequently required to confirm the diagnosis[4].

Pancreas transplant (PT) biopsy is a well-established procedure and remains the gold standard for differentiating causes of transplant failure, such as rejection, inflammation, or medication-associated toxicity[4,7]. Among these, rejection stands out as the most common cause of pancreatic graft loss[6]. The role of rejection and chronic injury in pancreas graft loss is still not well understood, largely because rejection rates tend to be underreported. This happens because many centers are cautious about performing pancreas biopsies, either due to the risk of complications or because of limited resources[8].

Rejection rates can vary depending on the immunosuppression protocols employed by each transplant center[4,9]. Notably, in simultaneous pancreas-kidney transplantation (SPK), diagnosing pancreatic rejection should not be based on kidney biopsy findings due to the low concordance between the two grafts. Recent studies have demonstrated this discrepancy, highlighting the necessity of pancreatic biopsy for accurate diagnosis[8].

According to the Scientific Registry of Transplant Recipients (SRTR) report for 2016–2017, the incidence of acute pancreatic rejection within the first year was 11.7% for pancreas after kidney (PAK), 19.2% for pancreas transplant alone (PTA), and 12.4% for SPK[8] figures that are far from negligible, further emphasizing the critical need for pancreatic biopsy.

In this study, we report a case series of pancreatic graft biopsies performed as part of the follow-up of patients undergoing SPK and PTA at a high-volume transplant center in Latin America. The primary objective is to evaluate the efficacy of biopsies as a diagnostic tool for detecting rejection and to analyze the potential complications associated with this procedure.

MATERIALS AND METHODS

A descriptive, observational, non-randomized, and retrospective study was conducted to analyze pancreatic biopsies performed in patients undergoing SPK and PTA between November 2003 and January 2025, at a high-

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complexity medical center in Córdoba, Argentina.

The study included all SPK and PTA patients who underwent pancreatic graft biopsy with documented records in their medical history. Cases without complete medical records were excluded.

The biopsy samples were consistently obtained by the same imaging specialist and processed by the same pathologist, both experts in transplantation.

Routine biopsies for the detection of subclinical rejection were not performed as part of patient follow-up; instead, biopsies were exclusively reserved for cases with suspected rejection.

Whenever an adequate access window was available, the biopsy was performed using an 18-gauge needle under ultrasound guidance with Doppler function to minimize the risk of vascular injury. In cases where this access was not feasible, the procedure was conducted under CT scan guidance, with laparoscopic biopsy reserved as a final option when both prior approaches failed.

Pancreatic biopsy samples were fixed in 10% neutral buffered formalin and subsequently processed for embedding in paraffin blocks. The descriptive analysis of rejection, when present, was performed according to the modified 2011 Banff classification, employing hematoxylin and eosin (H&E) staining. Both direct and indirect signs of cellular and humoral rejection were assessed, with immunohistochemical staining for the C4D marker conducted in all cases to evaluate humoral rejection, specifically examining its deposition in peritubular capillaries. In cases where humoral rejection was diagnosed based on C4D positivity, donor-specific antibody (DSA) testing was performed using the Luminex® assay.

Regarding the team's protocols, all transplants were performed using a duodenal anastomosis to the small intestine. The graft was positioned in the right flank, retrocolic, with the splenic vein anastomosed to the recipient's common iliac vein and the arterial anastomosis performed using a cuff of the donor's iliac arteries previously reconstructing the splenic and superior mesenteric artery into one single vessel.

All patients received induction therapy with a lymphocyte-depleting agent combined with corticosteroids, followed by a maintenance regimen based on calcineurin inhibitors and antimetabolites. Induction was achieved using rabbit antithymocyte globulin (Thymoglobulin®) and intravenous methylprednisolone administered during the perioperative period.

Maintenance immunosuppression consisted of tacrolimus, mycophenolate mofetil or mycophenolic acid, and oral corticosteroids. Tacrolimus levels were adjusted according to standard therapeutic ranges, with higher targets during the early postoperative period and progressive tapering thereafter. Corticosteroids were rapidly tapered during the first weeks until reaching low maintenance doses. Mycophenolate was administered in standard oral doses, with adjustments based on hematologic and gastrointestinal tolerance.

This triple-drug regimen was maintained in all recipients unless modifications were required due to adverse effects, cytopenias, or infectious complications. The immunosuppression strategy remained consistent throughout the study period, with minor adjustments reflecting institutional practice updates.

In cases of suspected acute rejection, indicated by elevated serum amylase and lipase levels, empirical treatment with intravenous methylprednisolone pulses was initiated at a dose of 250 mg twice daily for 5 days, while a concurrent pancreatic biopsy was performed to confirm the diagnosis. For cases where acute cellular rejection was confirmed through histopathological findings, the methylprednisolone regimen was continued until completion. If rejection was determined to be humoral, based on C4D positivity and/or the presence of donor-specific antibodies (DSA) detected by Luminex® assay, treatment was supplemented with thymoglobulin at a dose of 1.5 mg/kg/day for 3 days with adjustments according to lymphocyte counts and the patient's clinical response and lymphocyte counts.

For the purpose of the report, demographic and clinical variables were evaluated, including age at the time of transplantation, sex, body mass index (BMI), type and age of diabetes onset, disease duration, pre-transplant glycated hemoglobin levels, and type of transplantation.

The average amylase and lipase levels that prompted patients to undergo biopsy were analyzed. These levels were further examined using univariate and multivariate analysis to determine whether the enzyme elevations were associated with acute rejection and whether their increase correlated with the severity of rejection, as classified by the previously mentioned criteria. The sensitivity and specificity of both enzymes were determined, along with their ROC curves and cutoff values, thereby assessing their utility in the suspicion and diagnosis of acute rejection.

Each biopsy approach was evaluated, specifying the success rate based on whether the sample was conclusive for the corresponding analysis. Additionally, the percentage of biopsies confirming acute rejection was determined, followed by an in-depth classification of the rejection types according to histopathological findings.

Complications were assessed using the Clavien-Dindo classification, with the rejection rate specified based on the number of biopsy puncture attempts.

Data were collected and organized using a Microsoft Excel database. Absolute and relative frequencies were calculated for qualitative variables, while means and standard deviations, or medians with interquartile ranges (IQR), were estimated for quantitative variables as appropriate. For univariate analysis, the Chi-square test or Fisher's exact test was used for qualitative variables, and the student's t-test or

Mann-Whitney U test was applied for quantitative variables, depending on the data distribution. Multivariate analysis was then performed using logistic regression to identify independent factors associated with graft rejection. All analyses were conducted using SPSS v.25 for Windows, with a significance level of 0.05.

RESULTS

A total of 73 recipients underwent at least one pancreas allograft biopsy; however, 13 were excluded from the final analysis due to incomplete data or non-representative tissue samples, leaving 60 patients (56 SPK and 4 PTA) with 102 evaluable biopsies.Of these transplanted patients, 55% (n=56) underwent one biopsy, 21.6% (n=22) underwent two biopsies, and 23.4% (n=24) needed three or more biopsies, resulting in a total of 102 biopsies performed during the analyzed period. Of these biopsies, 93.3% (n=56) were performed in SPK patients, and 6.7% (n=4) in PTA patients.

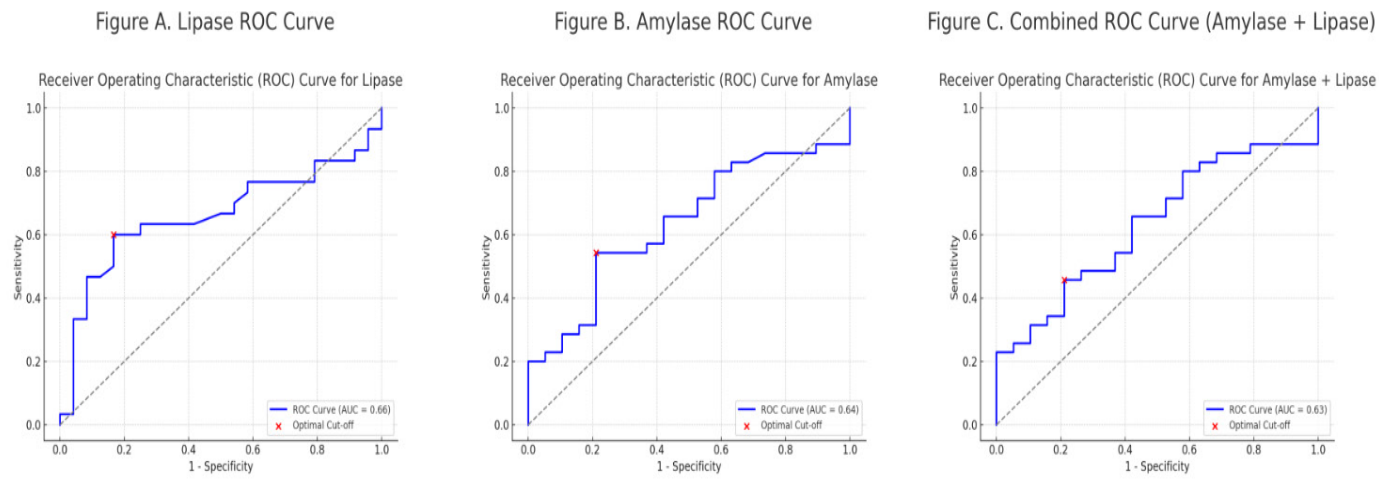
Table 1. Demographic variables.

Variables.	**n=73**
Age – median (IQR)	36.48 (31-46)
Gender	n (%)
Female	27 (36.7)
Male	46 (63.3)
Weight Status	**n (%)**
Underweight	4 (5)
Normal weight	46 (63.3)
Overweight	19 (26.7)
Obesity	4 (5)
Type of Diabetes	**n (%)**
Type 1	63 (86)
Type 2	10 (14)
Duration of Diabetes – median (IQR)	21 (17-26)
Glycated Hemoglobin – median (IQR)	8,3 (7,2-9,2)

All demographic variables are detailed in **table 1**.
**IQR: Interquartile range.*

Most biopsies were ultrasound-guided (89.2%, n=91), achieving adequate tissue sampling in 85% of cases. Biopsies guided by computed tomography (2%, n=2) and laparoscopic procedures (8.8%, n=9) had adequacy rates of 50% and 88.9%, respectively. A single puncture attempt was performed in 87.3% (n=89) of cases, while two puncture attempts were required in 9.8% (n=10), and three punctures in 2.9% (n=3).
The mean serum amylase level at the time of biopsy was 220 U/L (30 a 110 U/L), and the mean serum lipase level was 225 U/L (13 a 60 U/L). In univariate analysis, serum amylase and lipase levels were statistically significant predictors of pancreatic graft rejection, with p-values of 0.007 and 0.003, respectively. However, they did not demonstrate statistical significance in multivariate analysis and in predicting the severity of rejection based on the BANFF classification. Although each enzymatic elevation (amylase and lipase) was analyzed separately, due to the high concordance between the elevation of one and the other, the combined sensitivity of both was 45.7%, the specificity was 78.6%, and the area under the ROC curve was 0.633. **Fig 1**

Figure 1. A. ROC curve for serum lipase in predicting acute pancreatic graft rejection. The AUC was 0.66, indicating modest discriminative ability. The optimal cutoff achieved a balance between sensitivity and specificity, with lipase showing the highest. B. ROC curve for amylase, with an AUC of 0.64. While slightly lower than lipase, it still reflects limited diagnostic accuracy as a standalone biomarker. C. ROC curve for the combined model (amylase + lipase). The AUC was 0.63, suggesting that the combination of both enzymes did not enhance diagnostic performance and remains below the threshold of clinical utility.



Of the biopsies performed, 46.1% (n=47) did not report graft rejection. The characteristics of biopsies reporting rejection are detailed in **Table 2**.

Table 2. Characteristics of rejection biopsies.

n = 55	n (%)
Rejection.	**n= 55 (53,9)**
Cellular.	49 (89,1%)
Humoral.	6 (10,9%)
BANFF rejection rate.	**n (%)**
Mild.	32 (31,4)
Moderate.	16 (15,7)
Severe.	7 (6,9)

In this cohort, overall patient survival following SPK transplantation was 92.5% at 1 year, 88.1% at 3 years, and 80.3% at 5 years. Kidney graft survival was 90.7%, 82.9%, and 76.0% at the same time points, respectively, while pancreas graft survival was 78.2%, 70.1%, and 61.5%.

When outcomes were analyzed according to the performance of pancreas graft biopsy, recipients who underwent at least one biopsy (n=62) demonstrated comparable or slightly higher survival rates than those who did not. In the biopsy group, patient survival was 93.4% at 1 year and 84.6% at 5 years, with functioning kidney and pancreas grafts in 86.8% and 64.5% of cases, respectively, at 5 years. In contrast, recipients who were never biopsied (n=99) showed 91.2% patient survival and 78.9% kidney and 59.3% pancreas graft survival at 5 years. These findings indicate that clinically indicated pancreas allograft biopsies were not associated with inferior patient or graft survival. On the contrary, outcomes were broadly similar between groups, suggesting that biopsy can be safely performed when clinically indicated without detrimental long-term impact on graft function.

Post-biopsy complications were observed in 11.8% (n=12) of cases. Among patients who underwent a single puncture attempt, the complication rate was 9.2%, whereas those with multiple attempts experienced a significantly higher rate of 75%. A statistically significant association was identified between the number of puncture attempts and the occurrence of post-biopsy complications, with a p-value of 0.004.

According to the Clavien-Dindo classification of complications, 91.7% (n=11) identified in our study were classified as Grade I, and 8.3% as Grade II, with no major complications reported. The management of Grade II complications required the administration of one unit of red blood cells.

DISCUSSION

The first World Consensus Conference on Pancreas Transplantation highlighted the lack of conclusive evidence supporting the routine use of protocol biopsies in SPK. The performance of these biopsies remains largely center-specific and is typically part of immunological surveillance8,10. The consensus panel acknowledged the variability in clinical practice and the absence of standardized guidelines, reflecting ongoing uncertainties regarding the role of pancreatic biopsies in the context of suspected acute rejection.

In pancreas transplantation, there are no reliable, non-invasive laboratory or imaging studies for detecting graft rejection. Multiple laboratory markers are commonly used for patient monitoring, primarily pancreatic enzymes, glycated hemoglobin, and C-peptide. Despite their widespread use, pancreatic enzymes (amylase and lipase) have been widely questioned due to concerns regarding their sensitivity and specificity [11,12]. In our analysis, we found that serum amylase and lipase levels were statistically significant markers for pancreatic graft rejection (p=0.007 and p=0.003, respectively). However, these variables did not show statistical significance in the multivariate analysis and did not demonstrate a significant association with the severity of rejection according to the BANFF classification. [13,14]. The combined sensitivity of both enzymes was 45.7%, with a specificity of 78.6% and an area under the ROC curve of 0.633. This indicates that a significant number of patients with rejection may not present with elevations of this enzyme. This finding is consistent with previous studies that have evaluated the usefulness of protocol biopsies during the first-year post-transplant, as will be further discussed. This highlights the critical need to identify more precise and standardized markers, although pancreatic enzyme elevations will, for the time being, remain a key criterion for diagnostic suspicion.

This is why, at the slightest suspicion of rejection, percutaneous biopsy of the transplanted pancreas remains an essential tool for early diagnosis, allowing for the identification of both cellular and humoral components[7]. According to Fehrenbach et al.[7], tissue recovery rates in percutaneous biopsies range from 87% to 92%. In our experience, the rates of representative tissue were 85% and 98.1% for biopsies guided by ultrasound and surgery, respectively figures we consider highly satisfactory. For the CT-guided approach, however, our data revealed a concerning non-representative tissue rate of 50%. We believe this can be explained by the fact that CT-guided biopsy was performed in cases where ultrasound access was considered difficult or high-risk, meaning the CT approach was applied to already challenging patients, introducing a potential selection bias. Although CT guidance is more specific, it may remain challenging in these selected cases. In studies by other authors where CT guidance was routinely used, tissue recovery rates reached higher values, approaching 95%.

This underscores the problaby superiority of ultrasound in terms of yield and safety, although it is operator-dependent. We consider that it should be employed whenever the access window is deemed safe. At our center, percutaneous biopsies were consistently performed by a single experienced operator, which constitutes a critical factor in obtaining diagnostically adequate tissue samples. Several studies have demonstrated that operator expertise significantly influences diagnostic yield and complication rates, particularly in image-guided procedures[15].

For years, isolated renal biopsy was preferred in cases of suspected pancreatic rejection due to the widespread belief that rejection often occurs in both grafts simultaneously Additionally, renal biopsy was favored for its simplicity and lower risk of complications [10]. However, recent studies have shown a sensitivity of 60.53% and a specificity of 69.84% in predicting pancreatic rejection, underscoring its limited reliability. Therefore, despite the absence of standardized practical guidelines, pancreatic biopsy is now considered essential for accurate diagnosis and proper management of rejection[12-14,16].

Despite these advantages, percutaneous biopsies are not without risks. In our analysis, complications were observed in 11.8% of cases, primarily associated with the number of puncture attempts, which was statistically significant ($p=0.004$), consistent with the findings of Budhiraja et al[5]. In cases where more than one puncture attempt was necessary, we recorded a complication rate of up to 75%, highlighting the critical importance of imaging guidance and performing pancreatic biopsies under the supervision of experienced transplant specialists. Most complications (91.7%) were classified as Clavien-Dindo grade I, while 8.3% were grade II, requiring only a single transfusion of one unit of red blood cells, with a good response to conservative management. This highlights that, in expert hands, we deem it to be a safe and effective decision.

To compare our results with the most relevant literature, a report from the University of Maryland analyzed 63 recipients undergoing computed tomography-guided biopsy, reporting a similar complication rate of 8%, the majority classified as mild. The study concluded that, to minimize hemorrhagic and other complications, the pancreas should first be localized under ultrasound guidance, ensuring the identification of major blood vessels. Subsequently, puncture sites should be carefully selected to avoid large vessels and overlap with the small bowel

Beyond their diagnostic value, our data provide insight into the long-term outcomes of patients who underwent pancreas allograft biopsy. In this series, approximately one-third of SPK recipients required at least one biopsy during follow-up, most often due to suspected acute rejection. Notably, patient, kidney, and pancreas graft survival at 1, 3, and 5 years were comparable between biopsied and non-biopsied recipients, suggesting that the use of pancreas biopsy in the clinical decision-making process does not compromise long-term results. These observations reinforce that, within an experienced program, percutaneous pancreas graft biopsy performed under ultrasound or CT guidance is both effective and safe. The absence of an adverse impact on survival further supports its inclusion as part of the diagnostic workup for suspected graft dysfunction.

Nonetheless, these findings must be interpreted with caution. Because biopsies in our study were performed exclusively “for indication” and not as part of a systematic surveillance protocol, the analysis is subject to indication bias and immortal time bias. Therefore, it is not possible to infer a causal relationship between biopsy performance and improved outcomes. Rather, our results should be viewed as descriptive and hypothesis-generating, emphasizing that pancreas biopsy, when properly indicated and executed by experienced operators, remains a safe and indispensable diagnostic tool that does not negatively affect long-term graft or patient survival.

It is worth noting that some working groups perform routine biopsies at 1-, 3-, 6-, and 12-months post-transplant for the diagnosis of subclinical rejection, based on the premise that the first year is critical in terms of rejection rates. The percentage of subclinical rejection diagnosis in these cases reaches a concerning 43%, suggesting that this could potentially become as a routine practice. However, the authors concluded that long-term outcomes for patients with and without subclinical rejection are comparable. We believe that randomized studies with larger sample sizes are necessary to determine whether this protocol could be incorporated into specialized follow-up guidelines[10,20-22].

The observation that approximately 50% of biopsies performed due to elevated pancreatic enzymes did not reveal histological evidence of acute rejection underscores the diagnostic limitations of amylase and lipase when used in isolation. These findings are consistent with previous studies that have demonstrated the low specificity of these biomarkers in the context of pancreas transplantation. Budhiraja et al[10]. reported that a significant proportion of enzyme elevations were attributable to non-rejection-related phenomena, including mild pancreatitis, drug toxicity, or clinically nonsignificant fluctuations10. In our cohort, although serum amylase and lipase levels were statistically significant predictors in univariate analysis, they did not remain independently associated with rejection in the multivariate model. This reinforces the need to integrate additional clinical and biochemical parameters when deciding whether to proceed with biopsy. *Wan et al*[1]. also emphasize that, given the nonspecific nature of biochemical markers, graft biopsy remains a fundamental diagnostic tool for accurately assessing allograft dysfunction and guiding clinical decision-making[1]. This highlights the indispensable role of comprehensive clinical judgment and confirms the central importance of pancreatic biopsy in the workup of suspected rejection. Only two patients (3.3%) who initially showed no histological evidence of pancreatic rejection subsequently developed biopsy-proven acute pancreas rejection within the first six months of follow-up. Both responded favorably to corticosteroid therapy, with recovery of graft function. No patient in this subgroup developed kidney graft rejection.

Among the 47 biopsies without rejection, most (approximately two-thirds) demonstrated mild inflammatory or ischemic changes consistent with transient pancreatitis, periductal fibrosis, or drug-related injury (mainly tacrolimus or mycophenolate toxicity). In 8% of cases, histology was completely normal. In such instances, retrospective clinical review identified non-immunologic causes of enzyme elevation, including transient ischemia, early postoperative pancreatitis, gastrointestinal infection, or isolated biochemical fluctuation without clinical significance.

These observations highlight that even biopsies without rejection provided valuable diagnostic information, allowing clinicians to rule out immune-mediated injury and avoid unnecessary intensification of immunosuppression.

In conclusion, the elevation of pancreatic enzymes during the follow-up of patients undergoing SPK transplantation suggests the possibility of rejection, making pancreatic biopsy mandatory. In such cases, isolated renal biopsy appears insufficient due to its low sensitivity and specificity. Our report demonstrates that this procedure, when performed by experts and guided by Doppler ultrasound, achieves a high percentage of adequate tissue samples for analysis—provided that a suitable access window is available, blood vessels are properly identified, and intestinal interposition is avoided. There is an urgent need to incorporate more specific markers into follow-up protocols, along with randomized studies and large sample sizes, to support the development of standardized guidelines for the monitoring and management of rejection.

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