



Pancreas-Kidney Transplantation and the Evolution of Pancreatic Autoantibodies

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ABSTRACT

The recurrence or persistence of pancreatic autoantibodies after pancreas-kidney transplantation (PKT) is an intriguing finding. We prospectively analyzed 77 PKTs, searching for risk factors for the expression of these autoimmune markers and their impact on pancreas graft function. Among the 77 PKTs, 24.7% had 0 HLA matches, 20.8% displayed delayed graft function, and 14.3% had acute rejection episodes. Immunosuppression included antithymocyte globulin (ATG), tacrolimus, mycophenolate mofetil (MMF), and steroids. Sixty-five patients had both grafts functioning as a follow-up of more than 6 months. In 11 patients anti-glutamic acid decarboxylase (GAD) positivity persists ($n = 8$) or has recurred ($n = 3$), 4 of whom show increasing titers. Two patients maintain positive islet cell antibodies (ICA) and anti-GAD antibodies. The 9 patients positive for ICA included 2 who were negative before PKT and 7 who remain positive. The “positive” group (22 patients with positive ICA and/or anti-GAD) did not differ from the global group of 65 functioning PKT in terms of acute rejection episodes, HLA match, and steroid withdrawal. Among the positive patients, there were 2 with borderline glucose levels; however, among the entire “positive” group, the mean fasting glucose, HbA1c, and C-peptide measurements were not significantly different, when compared with the other 65 PKTs. In conclusion, pancreatic autoantibodies may be persistently positive or recur after PKT, despite appropriate immunosuppression. Its impact on long-term pancreas graft survival is unknown. We could not identify risk factors for their expression. An extended follow-up with monitoring and search for other risk factors may be necessary to increase our knowledge in this field.

THERE is substantial evidence for an autoimmune etiology of type 1 diabetes mellitus.¹ Pancreatic inflammatory infiltrates and circulating autoantibodies, such as anti-glutamic acid decarboxylase (GAD), islet cell antibodies (ICA), anti-tyrosine phosphatase (anti-IA2), and anti-insulin antibodies, have been documented at the onset of the disease. Several years after total endocrine pancreas loss, these autoantibodies persist or progressively fall to become undetectable. Measurement of autoantibodies is an easy, feasible technique that may be used to follow their evolution.

Pancreas-kidney transplantation (PKT) persists as the best treatment for type 1 diabetic patients with chronic renal failure.² After a successful PKT, graft loss due to acute rejection has become infrequent in recent years using current immunosuppressive protocols. Long-term graft loss may occur due to many factors, including chronic alloim-

mune responses (chronic rejection) and possibly also recurrence of autoimmunity.³ Some authors have observed an association between the recurrence of pancreatic autoantibodies and poor pancreas survival,^{4–9} or islet cell transplant survival.^{10,11}

Many PKT patients have undetectable titers of pancreatic autoantibodies; others show a progressive decrease in positive titers becoming negative, or maintaining stable level; some others, who were previously negative, become

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positive, despite maintained immunosuppression. Prospective monitoring of these serological markers may be useful; however, significance and impact on long-term pancreas graft survival are still unclear.

In this study we have presented our findings from a prospective monitoring of anti-GAD and ICA before and after PKT, searching for risk factors for the reappearance or maintenance of these autoantibodies, and looking for their possible consequences on pancreas graft function.

PATIENTS AND METHODS

From May 2000 to December 2007, we performed 77 PKTs from cadaveric donors in type 1 diabetic patients with chronic renal failure. The PKT technique consisted of a duodenopancreatic graft with enteric drainage, and venous anastomosis to the systemic circulation (common iliac vessels); the kidney graft was performed using the same technique as for a kidney alone transplantation.

The immunosuppressive protocol included induction with anti-thymocyte globulin (ATG-Fresenius-S) with tacrolimus (Tac), mycophenolate mofetil (MMF), and steroids as maintenance immunosuppression. Beside infection prophylaxis, all patients also received thrombosis prophylaxis with oral aspirin and subcutaneous low-weight heparin during the hospitalization; after discharge only oral aspirin was prescribed.

The diagnosis of acute pancreas rejection was inferred based on clinical, laboratory, and imaging signs, as well as kidney graft biopsy, which was always performed where there was pancreas and/or kidney dysfunction, after having ruled out other etiologies. We did not perform pancreas graft biopsies.

The permanent need for dialysis or insulin use was considered an index of graft loss. All patients were regularly followed at our outpatient care unit for 7 to 97 months.

RESULTS

The study group of 77 PKT patients included 50 women and 27 men of overall mean age at the time of transplantation of 33.5 ± 6.1 years (range, 23–48). Four of them were pre-emptive PKT, the others had pretransplantation dialysis times of 34 ± 26 months. The mean duration of type 1 diabetes was 22 ± 5 years. The mean HbA1c before transplantation was $8.5 \pm 1.6\%$. PKT was performed in the absence of any HLA match with the donor in 19 patients (24.7%).

The incidence of delayed renal graft function (transient need for dialysis during the first week after transplantation) was 20.8%. Insulin administration was discontinued at 1.5 ± 3.0 days after PKT. The median time from admission to discharge was 23 days, comprising a median stay in the intensive care unit of 2 days. The rate of postoperative surgical complications requiring surgical reinterventions was 33.8%, mostly due to bleeding, infection, or thrombosis.

Acute rejection was diagnosed in 11 patients (14.3%): in 3 patients both grafts were affected; in 6 patients only the kidney; and in 2 patients the diagnosis was presumed to affect only the pancreas graft. In 9 patients, the rejection episodes were efficiently treated with steroids only; in 1 patient with late, refractory, and recurrent rejection, OKT3 was used; in another patient it was a humoral rejection,

which reversed with plasmapheresis and intravenous immunoglobulin. Seventy-two PKT patients maintain functioning kidney grafts, with a mean serum creatinine level at the last visit of 1.12 ± 0.49 mg/dL (all but 2 were <1.6 mg/dL). The mean creatinine clearance was 75.1 ± 25.8 mL/min, without significant proteinuria (<0.5 g/d) except for 2 subjects. The 5 kidney graft losses were due to rejection ($n = 1$), infection ($n = 1$), and patient death ($n = 3$).

Sixty-five PKT patients have the pancreas and kidney functioning. At the last visit the fasting blood glucose of the 65 insulin-free patients was 81 ± 12 mg/dL; their HbA1c and C-peptide were $4.5 \pm 0.4\%$ and 3.4 ± 1.7 ng/mL, respectively. Twelve pancreas grafts were lost. The causes were infection ($n = 5$); thrombosis ($n = 4$); rejection ($n = 1$); bleeding ($n = 1$); and patient death ($n = 1$).

Twenty-five patients were converted from MMF to sirolimus (SRL), due to digestive intolerance in 24 and polyomavirus in 1 patient. In 49 patients (68.1%) it was possible to stop steroids. Five PKT patients died. Three patients died due to infection; 1 patient died due to cardiovascular disease at 34 months after transplantation and 5 months after he became dialysis and insulin-dependent, as a consequence of late, recurrent acute rejection. In another subject the cause of death is unknown. Patient survival rates at 1 and 5 years were 93% and 90%, respectively. Graft survival rates, also at 1 and 5 years, were for the kidney 91% and 88% and for the pancreas 86% and 81%, respectively. Among the 65 PKT patients with both organs functioning, 22 are now ICA-positive and/or anti-GAD-positive; thereafter denoted as the “positive” group, who were compared with the “global” group. Eleven patients showed positive anti-GAD: 3 were negative before PKT and 8 maintained positivity. Seven subjects show stable or decreasing titers, and 4 show increasing titers. Nine patients were positive for ICA (in 2 it was negative before PKT, 7 remain positive). Two subjects are positive for both autoantibodies. Six PKT patients who were anti-GAD-positive and 2 who were ICA-positive before transplantation are now negative. None of the 22 positive subjects needs insulin and, at the moment, they have no signs of pancreatic graft dysfunction or rejection. Only 2 of these (9.1%) have had early acute pancreas rejection with good responses to therapy.

Nine of the 22 (36.4%) had no HLA match, an incidence slightly higher than in the global group, but without statistical significance ($P =$ not significant [NS]). Steroid withdrawal in the positive group was possible in 68.2% (15/22), which was similar to the global group. Seven of the 22 (31.8%) were converted from MMF to SRL, almost the same as among the global group. Also in terms of Tac levels (7.3 ng/mL in the “positive” vs 7.6 ng/mL in the “global”), SRL levels (5.4 ng/mL in the “positive” vs 5.2 ng/mL in the “global”), or MMF daily dose (1000 mg in the “positive” vs 1250 mg in the “global”), the 2 groups did not differ.

Two positive patients show “normal high” glucose levels and HbA1c $>5\%$, 1 of whom has the lowest C-peptide (0.7 ng/mL), which is still within the normal range. However, the mean fasting glucose and HbA1c values of all of the positive

group were not different from the values of the global group.

DISCUSSION

Pancreatic autoantibodies may in fact remain positive among PKT patients, as we have observed. Their evolution is uncertain. The conditions that may contribute to persistent positivity after transplantation under apparently sufficient immunosuppression are not known. Possible risk factors for the activation of the immune (at least alloimmune) response were analyzed in this study. Our results did not show any differences in terms of acute rejection episodes, type and dosage of immunosuppressants, and number of HLA matches between the positive and the other patients. Also, among the positive patients we have not observed a negative impact on pancreas graft function or poor glycemic control. Some authors have reported a correlation between reduction of immunosuppression and pancreatic autoantibody relapse and graft loss.^{12,13} We did not observe this association; like other investigators¹⁴ we could not correlate its positivity and pancreas graft loss. Without evident signs of acute rejection or uncontrolled alloimmune activity, there seems to be no reason to increase immunosuppression in these patients.

Long-term consequences of pancreatic autoimmune markers for pancreas graft function and survival are still unclear. We think that it is advisable to expand the follow-up and maintain careful monitoring of autoantibodies and pancreas function, meanwhile searching for other factors that may influence autoimmune expression.

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