Autoimmune diabetes recurrence should be routinely monitored after pancreas transplantation

La Salete Martins

Autoimmune type 1 diabetes recurrence in pancreas grafts was first described 30 years ago, but it is not yet completely understood. In fact, the number of transplants affected and possibly lost due to this disease may be falsely low. There may be insufficient awareness to this entity by clinicians, leading to underdiagnosis. Some authors estimate that half of the immunological losses in pancreas transplantation are due to autoimmunity. Pancreas biopsy is the gold standard for the definitive diagnosis. However, as an invasive procedure, it is not the ideal approach to screen the disease. Pancreatic autoantibodies which may be detected early before graft dysfunction, when searched for, are probably the best initial tool to establish the diagnosis. The purpose of this review is to revisit the autoimmune aspects of type 1 diabetes and to analyse data about the identified autoantibodies, as serological markers of the disease. Therapeutic strategies used to control the disease, though with unsatisfactory results, are also addressed. In addition, the author's own experience with the prospective monitoring of pancreatic autoantibodies after transplantation and its correlation with graft outcome will be discussed.

Key words: Autoantibodies; Autoimmune type 1 diabetes; Pancreas transplantation; Type 1 diabetes recurrence

Core tip: Recurrence of pancreatic autoantibodies after kidney-pancreas transplantation is a disturbing finding. It was estimated that half of the immunological losses of pancreas grafts may be due to autoimmunity. There is a rising investigational effort concerning this issue. At our unit, we have designed a protocol of prospective monitoring of pancreatic autoantibodies after transplantation. In our experience, patients with positive pancreatic autoantibodies, compared to negative patients, were more likely to present higher HbA1c and lower C-peptide levels. A review of the most important publications in this field, and about the interest of pancreatic autoantibodies monitoring after transplantation, was made.

© 2014 Baishideng Publishing Group Inc. All rights reserved.
pancreas grafts with HLA-mismatches with the donor and maintaining standard immunosuppression\textsuperscript{9}.

There is a recognized genetic susceptibility for DM1. The disease is strongly associated with \textit{HLA} genes, specifically with alleles DR3 and DR4\textsuperscript{5,6}, with polymorphisms of the proinsulin/insulin gene\textsuperscript{17}; and with the PTP gene (\textit{PTPN22}), a gene coding for a lymphocyte-specific tyrosine phosphatase\textsuperscript{8}. However, only about 50\% of HLA identical twins inheriting alleles DR3 and/or DR4 develop the disease\textsuperscript{1,9}. It means that inheritance of the \textit{HLA} gene is not a sufficient condition and susceptibility is most certainly polygenic.

It is not known which individuals are at higher risk for DM1 recurrence on pancreas graft and what are the important risk factors for the disease. Additionally, there is also no consensus about the best screening tests to identify patients at risk.

**DIAGNOSIS OF AUTOIMMUNE DIABETES RECURRENCE ON PANCREAS GRAFTS**

A pancreas graft biopsy showing an inflammatory T-cell infiltrate, specifically targeting the beta-cells (aspect designated as “insulitis”) and sparing the exocrine tissue, remains the gold-standard for the diagnosis of DM1 recurrence\textsuperscript{40}. However, it is not easy to justify such an invasive procedure, carrying a non negligible risk of complications, in patients without a misfunctioning pancreas graft; or, at least, without reliable data favouring the hypothesis of reactivated autoimmunity.

Serological markers of the autoimmune process, the islet cell autoantibodies (ICA)\textsuperscript{10} have been proposed as a basic tool, the first screening test to identify the activity of autoimmune disease. The anti-glutamic acid decarboxylase (anti-GAD antibodies)\textsuperscript{11,12}, the anti-insulin antibodies (IAA)\textsuperscript{13,14}, the anti-IA2 (anti-tyrosine phosphatase) antibodies\textsuperscript{15,16}; and the most recently described anti-ZnT8 (cation efflux zinc transporter) antibodies\textsuperscript{17,18}, have also been identified as autoimmune markers of DM1. The positivity for these immune humoral markers is considered a good predictor of the enhancement of autoimmune diabetes. The association of several markers (two or more) increases its predictive value\textsuperscript{19}. As yet, pancreas biopsy is the confirmatory procedure when suspecting for recurrence on pancreas graft.

There is some controversy about the real role of these autoantibodies: do they have a direct participation in the disease? Or are they surrogate markers, merely testifying the lesion? Although the pancreatic autoantibodies were not detected in a recent case documented with insulitis in the biopsy\textsuperscript{10,13}, they are usually present in the vast majority of the cases confirmed by biopsy.

The new onset or rising levels of these autoantibodies in pancreas transplant patients has been pointed out as a serious indicator of recurrence and progression of the disease. In fact, several studies reported worse pancreas outcome in patients with these humoral markers\textsuperscript{20,21-23}. It has been suggested that half of the immune losses of pancreas grafts may be due to autoimmunity\textsuperscript{24}. Based on these data, monitoring of pancreatic autoantibodies has been recommended in all pancreas transplants\textsuperscript{25} as a primary test to identify patients at risk for autoimmune graft loss. My personal opinion is concordant with these authors, stating that the disease may currently remain underdiagnosed. This may be the cause of pancreas graft failure in some cases with unclear etiology, probably because this is not sufficiently investigated.

**IMMUNOSUPPRESSION AND AUTOIMMUNITY**

Immunosuppressive protocols designed to prevent rejection in the pancreas transplant are not capable of containing autoimmunity\textsuperscript{21}. This is a disturbing finding in organ transplantation. Remembering autoimmune disorders affecting the kidney, such as ANCA-associated vasculitis or lupus, they may relapse after kidney transplantation despite apparently adequate immunosuppression to control alloimmunity. One condition in kidney transplantation, which is quite similar to the pancreatic autoimmunity recurrence, is that observed in some patients with Alport syndrome: they may develop anti-glomerular basement membrane disease post-transplantation, after a new exposition to glomerular basement membrane antigens (type 4 collagen antigens), for which they were natively defective. Immune attack against the newly presented beta-cells may occur after a pancreas transplant.

The Miami group has tried to treat autoimmune relapse in pancreas transplants with anti-lymphocyte (anti-B and/or anti-T cell) therapies\textsuperscript{21-23}. After a transient response in a few cases, autoimmune activity has recurred within a short period of time. At the time of the second recurrence they were able to identify the same clone of autoreactive GAD-specific T cells which has been found in the first recurrence. Pancreatic autoantibodies followed the reappearance of the T cells, with a new rise\textsuperscript{23}. Therefore, it seems that immunosuppressive agents available at the moment cannot prevent this immune memory response. To date, there are no studies reporting effective and sustained treatment of pancreatic autoimmunity in DM1 patients with diabetes recurrence.

Efforts are needed to find therapeutic strategies to control this process. Can protocols used in kidney transplant hypersensitized patients be advantageous? Combined therapies, like plasmapheresis, immunoglobulin and rituximab have been successfully used in kidney transplants with HLA donor-specific antibodies; and also in systemic autoimmune diseases, such as lupus, with severe expression. The results from trials using new drugs (abatacept, etanercept, teplizumab, rituximab) have failed to prove long lived efficacy in native pancreas after DM1 onset\textsuperscript{24}. However, intervention after clinical disease (tertiary intervention) may be too late, since overt disease corresponds to extensive beta-cell destruction. The most promising long-term results were achieved with hematopoietic stem-cell transplantation, in patients pre-
senting autoimmune markers, but before clinical diabetes (secondary intervention)[24]. Another serious worry are the important side effects of each drug. Toxicities and efficacy, if used in combination, remain to be assessed. We have learned the benefits of the association of complimentary therapies from the oncology research, allowing the use of smaller doses, with fewer side effects and with gains in terms of efficacy. Could this be an efficient strategy in pancreas transplant patients with recurrent autoimmune diabetes?

**OUR OWN EXPERIENCE**

We have designed a prospective monitoring protocol of pancreatic autoantibodies after pancreas-kidney transplantation at our unit. Anti-GAD, IAA and ICA were analysed before or on admission for transplantation, at 6 and 12 mo, and at least once a year thereafter. In a cohort of 135 patients[25], 34.5% were positive for any of these antibodies before surgery, anti-GAD being the most prevalent (> 20%). Nearly half of these became negative within months or years, but in some others (previously negative) we verified a new appearance of anti-GAD antibodies. After a mean time of 6 years (ranging from 1 to 12 years), among the 78% of the patients with functioning pancreas, 44% are positive for at least one autoantibody. Anti-GAD remain the most common (31%). The frequency of patients with IAA before transplantation was surprisingly low, 3%, considering that patients under exogenous insulin may present anti-insulin antibodies. At our last follow-up the prevalence of IAA-positive is three-fold higher and none of the patients was under exogenous insulin. ICA, present in 10% of the patients before transplantation, tended to disappear and there was no new onset of these antibodies. Less than 3% maintain ICA positive now. In 10% of the patients more than one pancreatic autoantibody was present.

Pancreas graft survival was not significantly different in the group of patients with some positive pancreatic autoantibody, compared to the patients who were negative for these autoantibodies. The immunosuppression used in the positive and in the negative patients did not differ (tacrolimus and mycophenolic acid mostly used) and the frequency of patients withdrawn from steroids was also similar in both groups.

Positive patients for any pancreatic autoantibody tended to have a higher HLA-match with the donor, though not reaching statistical significance.

Concerning the glycemic control, our data are not so tranquilizing. The group of patients with at least one positive pancreatic autoantibody, compared to negative patients, was more likely to have higher HbA1c and lower C-peptide levels and this difference was statistically significant. And, more important, our results showed a more than 5 times higher probability to find positive autoimmunity among the pancreas transplants with normal-high HbA1c. Kidney graft function was similar in both groups, with or without pancreatic antibodies, which strengths the argument that decline in glycemic control was not due to alloimmunity. In a former report from our centre[26], analysing the glycemic profile in both groups (positive and negative for pancreatic autoantibodies), the difference did not (yet) reach statistical significance.

In fact, in this former study, a few number of patients with positive antibodies showed normal-high fasting glucose levels and the lowest C-peptide. Comparing results from our former study to the more recent one, it probably means that we are facing an evolutive process. The number of patients has almost doubled and the follow-up period is now much longer, and we could now associate pancreatic autoimmunity with less favourable glycemic profile. It will be interesting to analyse data with a more extended follow-up.

**CONCLUSION**

In conclusion, we think it is advisable to routinely monitor pancreatic autoantibodies after transplantation. There is substantial evidence that DM1 can reappear after pancreas transplantation, but may have been underdiagnosed for the last decades[21,27], mainly because it is a forgotten question. The awareness of the entity will certainly lead the majority of pancreas transplant units to search for the disease and to prospectively assess these antibodies. Pancreas graft biopsy remains the gold standard for the diagnosis of DM1 recurrence but, as it is an invasive procedure, it is not the ideal as a screening methodology, without other clinical or analytical (metabolic or immunological) data. On the other hand, pancreas biopsy only after impaired endocrine function is most of the times too late. Antibody monitoring is a non-invasive basic screening test, available in most units, and may bring the necessary information to proceed to pancreas biopsy before dysfunction. Islet autoantibodies are currently the most robust biomarker of DM1[28].

Additionally, pancreatic autoantibodies assessment may be of interest in other areas. The authors of a very recent study[29] have also proposed the use of the GAD-autoantibody status before pancreas-kidney transplantation as a guide to choose the kind of prophylactic antibody induction.

Positivity for pancreatic autoantibodies after transplantation may never occur, or it may be intermittent or persistent, not always correlated with graft function, at least in a short period of time. However, beta-cell destruction was documented in other cases and autoantibody rising may have preceded in several years the graft dysfunction, has it been searched for. An early diagnosis gives the clinician more time for some kind of intervention.

Another critical point is the lack of effectiveness of the treatments tried so far in DM1 patients. The recognition of the role of islet autoreactive CD4+ T cells[22,23] and CD8+ T cells[27,34] on beta-cell destruction, as well as all the targets of humoral activity[30], may lead to other treatment opportunities. Novel therapies, namely targeting proinsulin-reactive CD8+ T cells, were recently pro-
posed as a potential therapeutic approach.

In the meantime, we must improve our ability to make an early diagnosis and to increase our knowledge about all the processes of the disease. These may be some of the missing steps to find the most advantageous strategy to treat, or even to prevent, the autoimmune diabetes recurrence in pancreas grafts.

REFERENCES

Martins LS. Autoimmune diabetes recurrence in pancreas transplants


P- Reviewer: Marek-Trzonkowska N, Senior PA
S- Editor: Wen LL
L- Editor: A
E- Editor: Liu SQ