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Virtual HLA Crossmatching as a Means to Safely Expedite Transplantation of Imported Pancreata

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Abstract

Background—Imported pancreata accumulate cold ischemia time (CIT), limiting utilization and worsening outcomes. Flow cytometric crossmatching (FXM) is a standard method to assess recipient and donor compatibility, but can prolong CIT. Single antigen bead assays allow for detection of recipient donor-specific HLA antibodies, enabling prediction of compatibility through a ‘virtual crossmatch’ (VXM). This study investigates the utility and outcomes of VXM following transplantation of imported pancreata.

Methods—We retrospectively compared outcomes of 153 patients undergoing pancreas transplantation at our institution over a 3.5 year period.

Results—Three patient groups were analyzed based on geographic source of the pancreas graft and the type of prospective XM performed: 1) Imported VXM only, n=39, 2) Imported VXM + FXM, n=12, and 3) Local VXM + FXM, n=102. There were no episodes of hyperacute rejection and 1 episode of early antibody mediated rejection (<90 days) in the imported VXM group. Death-censored graft survival, patient survival, and rejection rates were comparable among the recipient groups. For pancreata imported from UNOS regions 3 and 4, proceeding to surgery without a FXM reduced CIT by 5.1h ($p < 0.001$). The time from organ arrival at the hospital to operation start was significantly shorter in the VXM only group compared to the VXM+FXM group ($p < 0.001$).

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Conclusions—VXM helps minimize CIT without increasing rejection or adversely affecting graft survival making it a viable method to increase pancreas graft utilization across distant organ sharing regions.

Introduction

Pancreas transplantation increases longevity and improves the quality of life in diabetic patients (1–4). Successful pancreas transplants provide long-term insulin independence, which improves glycemic control without hypoglycemic risk while preventing further diabetes-related complications (5). As in kidney transplantation, donor and recipient immunocompatibility are assessed before transplantation. The standard method for evaluating compatibility is the flow cytometric crossmatch (FXM), which detects the presence of donor specific antibody (DSA) by assessing the binding of recipient antibody to donor lymphocytes using indirect immunofluorescence and flow cytometric analysis (6). Although effective, a FXM requires the availability of viable donor cells and is time consuming, typically requiring 3–4 hours to complete once donor samples arrive in the lab (7).

Pancreata are often shared between centers across long distances and are transported from the donor hospital to the recipient transplant center. In the US, transportation usually occurs via commercial airlines and can often take 12–18 hours or more from the time of cross-clamp to arrival at the recipient center. Due to the extended transport times required for shipping pancreata across long distances, such as exists in the US, one of the principal limitations to utilizing these organs is the accumulation of cold ischemia time (CIT) during shipment. It is well understood that the pancreas is susceptible to ischemia-reperfusion injury and that longer CITs are associated with worse surgical outcomes (8–10). The additional 3–4 hours required to complete FXM testing further extends the CIT compounding the potentially deleterious effects on the pancreas graft. Furthermore, over the last decade, pancreas graft utilization across the US has declined (11), in part, due to fears of poor outcomes related to long CITs. In fact, average CITs for completed pancreas transplants in the US have declined suggesting less tolerance for longer CITs (11). These factors have stimulated us to consider using alternative XM methods with the goal of reducing CIT.

Solid phase assays using HLA single antigen bead (SAB) assays have been used to identify and semi-quantitate recipient anti-HLA antibodies with high sensitivity and specificity (12). With this information, a recipient's compatibility with a prospective donor can be predicted using a "virtual crossmatch" (VXM), potentially eliminating the need for a cell-based crossmatch (6). Recipients with high levels of pretransplant anti-HLA antibodies targeted to antigens of the donor are likely to exhibit early rejection or premature graft failure while recipients with low levels of pretransplant DSA may be less likely to experience early immunological graft injury (12). Multiple studies indicate that VXM using Luminex SAB assays are more sensitive and specific for detecting DSA than FXM (13). In recent years, VXM has provided the basis for improved national allocation of compatible kidneys into sensitized recipients through paired kidney exchanges (14,15). VXM has also been shown to decrease wait times for heart transplantation without increasing the risk of cellular rejection,

antibody mediated rejection, or mortality (16). Moreover, SAB testing may be associated with lower rates of false positive results compared to FXM (17). Reasons for this may be due to detection of donor autoantibodies, non-HLA antibodies as well as the binding of Fc receptors that are encountered in a FXM assay that are not encountered in SAB assays (18). At some centers, a positive FXM in patients with little or no DSA may be considered a contraindication to transplantation, so a false positive result can deny a patient access to a compatible transplant (19,20).

The potential benefit of VXM is its ability to provide an assessment of donor-recipient compatibility without subjecting the imported pancreas to additional CIT required for FXM testing. The ability to reduce CIT may not only contribute to improved outcomes by reducing ischemia-reperfusion injury, but also may increase the overall number of pancreata accepted and transplanted. However, the impact of using VXM in the setting of imported pancreas transplants and its potential benefits in reducing CIT have not been reported. Therefore, in this study we evaluated the outcomes of using VXM as the only pretransplant compatibility test to decide whether to proceed with transplanting imported pancreata. We demonstrate that VXM is a time-saving and safe method allowing transplantation of imported pancreata, and avoids the need to wait for FXM results in most cases.

METHODS

Study Design

We retrospectively reviewed the charts of solitary pancreas (SP) and simultaneous pancreas-kidney (SPK) transplants performed from June 2010 to December 2013 at the University of Wisconsin Hospital and Clinics (n=153 patients). We chose this beginning point based on when Luminex SAB and FXM were routinely used for HLA antibody identification and DSA detection (21). The end point for this study was June 30 2014, providing between six months and four years of follow-up on each patient.

Comparisons were made between three recipient groups transplanted concurrently as follows, based on the pancreas they received: 1) imported pancreas, VXM only prior to transplantation (n=39), 2) imported pancreas, VXM and FXM prior to transplantation (n=12), and 3) local pancreas, VXM and FXM prior to transplantation (n=102) (Figure 1). For all local donors a pretransplant FXM was performed in addition to a VXM. The reason for this is that in our center, with a single center organ procurement organization (OPO), a FXM does not delay the transplant of a locally procured pancreas. A FXM was performed pretransplant primarily if recipients had antibodies against HLA-DP and/or HLA-DQA1 and if the imported donors were not typed for these loci, as a VXM would be considered incomplete if the full donor HLA typing including HLA-DP and -DQ is not available (22). Outcomes of interest were graft survival, patient survival, death-censored graft survival, biopsy-proven rejection, CIT and the time from arrival of the imported graft to OR start time.

Crossmatching

For Luminex SAB analysis each recipient's serum was analyzed for HLA antibodies using Class I and Class II beads (LabScreen® One Lambda, Inc. Canoga Park, CA, USA) on a Luminex instrument. Local donors were routinely typed for HLA-A, B, C, DRB1, DQB1, DQA1, and DPB1. Recipients were typed for HLA-A, B, C, DRB1, and DQB1; typing for HLA-DQA1 and DPB1 was performed when needed to confirm anti-DQA1 and DPB1 antibody specificities. HLA antibody specificities were assigned based on patterns of epitope specificity, background levels, and individual bead performance consistent with the recently published consensus guidelines of HLA antibody testing (23). DSA determinations were made based on HLA antibody specificities as previously described (24,25). A negative virtual crossmatch was assigned when every single DSA had an MFI less than 1000.

Three-color flow cytometric crossmatches (FXM) were performed on Ficoll-hypaque purified mononuclear cells using standard methods (26). T and B cells were identified by staining with PerCP-anti-CD3 and PE-anti-CD19, respectively. The FITC-conjugated goat-anti-human IgG (γ -chain specific) was obtained from Jackson ImmunoResearch (West Grove, PA). A positive FXM was defined as a median channel shift that is at least three standard deviations greater than the signals obtained from at least 25 samples stained with normal human serum.

Surgical Methods

All pancreas transplants were performed with systemic venous drainage and enteric drainage of exocrine secretions via side-to-side duodeno-jejunostomy. Recipients received anti-thymocyte globulin, alemtuzumab or basiliximab for induction antibody therapy in conjunction with tacrolimus, mycophenolate mofetil and steroids for maintenance therapy.

Pancreas graft failure was defined as pancreatectomy, relisting for pancreas transplant, or return to any exogenous insulin therapy for greater than 3 months. All rejection episodes were confirmed by needle core biopsy and graded according to the 2011 Banff criteria (27)

Data Analysis

Categorical variables were compared between groups using Fisher's exact test and continuous variables were compared using student's t-tests. Graft survival, patient survival, death-censored graft survival, and rejection rates were determined by time-to-event analysis. Biopsy proven acute rejection rates including rates of acute cellular and acute antibody mediated rejection (ACR and AMR, respectively) were determined. All analyses were performed using either SPSS v22 (IBM, Armonk NY) or SAS statistical software version 9.2 (SAS Institute Inc., Cary NC).

This study was conducted under approval of the UW School of Medicine and Public Health IRB (# M-2010-1361) and was in compliance with HIPAA.

RESULTS

Over a 3.5 year period 51 pancreata were concurrently transplanted using imported organs: 39 transplants were performed based solely on VXM results and 12 based on FXM and

VXM results in the case of incomplete donor typing (Figure 1). Transplants that began based solely on a VXM had a FXM performed retrospectively, all of which were negative and thus the VXM was predictive of the FXM in this series. Donor and recipient demographics, immunosuppression regimens and surgical parameters were comparable between groups with the exception of donor ethnicity, transplantation type, number of previous transplants, sensitization, and the use of depleting or non-depleting induction therapy (Tables 1 and 2). As one might expect, donors of imported organs were more ethnically diverse than the local donors (UW donor service area, Table 1). Imported pancreata were primarily SP transplants while locally procured pancreata were primarily SPK transplants (imported VXM only, 87% SP vs. imported VXM + FXM, 100% SP vs. local, 13% SP, $p < 0.001$) (Table 2). The proportion of SP transplants in the imported vs. local group reflect institutional policy and previously existing allocation rules in the US. Patients in the imported groups were more likely to have had previous transplants and were more sensitized than those in the local group, having a higher average peak panel reactive antibody (PRA) (Table 2). The patients in the imported groups were also more likely to have DSA present pretransplant (imported VXM only, 41% vs. imported VXM + FXM, 67% vs. local, 7%, $p < 0.001$). The imported groups did not differ from each other with respect to pretransplant PRA or DSA (Table 2). Based on the higher proportion of SP and prior transplants in the imported groups, as well as higher degree of sensitization, the imported groups represented higher immunological risk transplants (28). Because of this, the imported groups were more likely to have had a T-cell depleting drug (Anti-thymocyte globulin or Alemtuzumab) for induction rather than the non-T-cell depleting antibody Basiliximab (Table 2).

A major concern with forgoing a FXM prior to transplantation and relying solely on VXM is the possibility of early hyperacute or antibody mediated rejection despite a negative VXM. To assess this risk we compared groups for occurrences of hyperacute rejection, or ACR, AMR, and mixed rejection by 90 days posttransplant. In the imported VXM only group we observed no episodes of hyperacute rejection or graft thrombosis, which can be the sequela of severe immunological injury. Within 90 d posttransplant, groups did not differ in the rates of biopsy-proven ACR, AMR, or mixed rejections (Table 3). The imported VXM only recipients did experience a higher rate of ACR at the end point of the study in comparison to recipients transplanted with locally procured pancreata; however, the ACR rate in the imported VXM group was not significantly different from the imported VXM + FXM group (Table 3). AMR rates did not differ among groups at 90 d or at the terminal point of the study (Table 3), despite a higher proportion of SP transplants and sensitized patients in the imported groups.

Given the known higher risk of rejection seen in SP transplants in comparison to SPKs (5,29), we compared rejection rates of like-transplants among the three groups. When comparing SPs to SPs and SPKs to SPKs, the rates of ACR, AMR, and mixed rejection did not differ between groups at 90 d or at the end of the study (Table 3).

Comparing patient survival among groups showed no statistically significant differences at one year (imported VXM only, 89.7% vs. imported VXM + FXM, 100% vs. local, 96.9%, $p = 0.16$); however, there were several deaths with functioning grafts in the imported VXM only group that were unrelated to the transplant (1 suicide, 1 motor vehicle accident, 1

unknown cause of death). Death-censored graft survival was not different among the groups at one year (imported VXM only, 94.9% vs. imported VXM + FXM, 83.3% vs. local, 89.2%, $p = 0.45$) (Figure 2).

As expected, CIT was shorter in the local group than in either of the imported groups and 1.5 h shorter in the imported VXM only group than in the imported VXM+FXM group (imported VXM only, 16.0 h vs. imported VXM + FXM, 17.5 h vs. local, 13.2 h, $p < 0.001$) (Figure 3). Given the variability in origins of imported pancreata, CIT was compared among pancreata originating from similar destinations (Figure 4). UNOS regions were combined for purposes of statistical analysis. For example, regions nearest to our center, namely regions 7, 8, and 10 (midwest), were grouped together given the expected shorter transportation time. Indeed, mean CIT for pancreata from these regions was 13.3 hours and was similar to locally procured pancreata from our own donor service area. Pancreata imported from UNOS regions 5 and 6 (west coast) averaged 17.9 hours, significantly longer than from regions 7, 8, and 10, but did not differ between imported VXM only and imported VXM + FXM groups. In contrast, for pancreata imported from UNOS regions 3 and 4 (south) proceeding to surgery without a pretransplant FXM saved on average 5.1 hours (95% CI [3.25, 6.98]) ($p = 0.0001$). The average CIT for pancreata imported from these southern regions without a FXM was 15.6 h compared to 20.7 h with a pretransplant FXM ($p = 0.0001$). Strikingly, we did not import any pancreata from UNOS regions 1, 2, 9, and 11 (northeast) during this period in which we needed to wait for a pretransplant FXM. The six pancreata in this study that were imported from northeast regions were transplanted with only a VXM pretransplant. These data suggest that our ability to forgo pretransplant FXM impacted our ability to import pancreata from additional UNOS regions (ie. the northeast) and reduce CIT from other UNOS regions (ie. the south).

We also sought to compare the time from organ arrival at our center to OR start time between imported pancreata transplanted after only a VXM vs. those transplanted after a VXM and FXM performed after organ arrival. We found that for organs that proceeded to transplantation without a pretransplant FXM, the mean time from arrival to OR was over 2.5 hours shorter than those that went to the OR after a FXM (imported VXM only: 149.9 min vs. imported VXM+FXM: 296.3 min., $p < 0.001$) (Figure 5).

DISCUSSION

The donor-recipient crossmatch allows a transplant center to predict the risk of hyperacute rejection for a particular transplant. Traditionally, centers have relied on NIH CDC crossmatching or more recently FXM, but for imported organs a cross-match can add a significant amount of CIT to the transplanted organ. Performing a VXM can potentially eliminate the need for performing a cell-based cross-match (13). However, using VXM as the sole method for determining histocompatibility has not been reported in the setting of pancreas transplantation. By eliminating the need for a cell-based cross-match before transplantation, VXM may serve as an effective tool to decrease CIT for imported pancreata, thereby facilitating both the transplantation of highly sensitized patients and potentially increasing pancreas utilization. Here, we demonstrate that using VXM alone for pancreas transplantation is safe and did not negatively affect short term outcomes. We did not observe

any episodes of hyperacute rejection in this series and both the imported groups and the local group did not differ in AMR rates at both 90d and at the terminal endpoint in the study. We did see a numerical, but not statistically significant, increase in ACR in both imported groups compared to the local group at the terminal point in the study, but not between imported groups. We attribute this to a preponderance of SP and prior transplants as well as more sensitized patients in the imported groups, characteristics which are known to be associated with higher rates of rejection (29). It is well known that ACR rates are in part related to factors such as immunosuppressive medication adherence and dose modifications due to infections and side effects, factors not directly related to donor-recipient immunologic compatibility. Nonetheless, to partially control for differences in immunological risk between SP and SPK transplants we performed a comparison between groups stratified by transplant type. Comparing SP outcomes and SPK outcomes separately among all three groups showed no difference in the rates of ACR, AMR, and mixed rejection.

Observing similar rejection rates among the groups, together with the fact that the imported groups were significantly more sensitized than the local group, further strengthens the conclusions that VXM in pancreas transplantation is an acceptable practice. Indeed, prior studies have demonstrated that VXM can facilitate transplantation of highly sensitized kidney transplant recipients by allowing the identification of prohibited HLA antigens thereby increasing the likelihood of a donor offer having a negative FXM (7,14,15). In the setting of kidney transplantation, the listing of prohibited HLA antigens and calculated PRA (cPRA) for sensitized kidney transplant candidates in UNOS after 2009 led to an increase in the proportion of transplants to 80+% cPRA recipients from 7% to over 15%, and a ten-fold decrease in positive cross-matches (15). Furthermore, SAB-based listing of prohibited HLA antigens has greatly helped facilitate the national implementation of kidney paired exchange programs (14). Thus, using VXM may facilitate the transplantation of highly sensitized pancreas transplant candidates while providing safety with an expectation of low early rejection rates. As ongoing CMS discussions address the allowance of VXM for kidney and pancreas transplants, the safety and efficacy of VXM shown by our data support the implementation of such protocols.

While we believe our data supports the safe use of VXM for pancreas transplants we want to stress the importance of making these decisions in conjunction with a highly trained HLA lab. We are also not advocating for only a VXM to be used without a FXM where there is incomplete donor typing. For example, during this study, HLA-DP typing of donors was not required by UNOS and was therefore not consistently available. When the donor has not been HLA-DP typed, it makes it difficult to comprehensively assess the true likelihood of a positive crossmatch when a recipient has high or equivocal DP antibody levels (eg MFI >1000). If a recipient is known to have high DP antibodies and donor DP typing is not available, then it would be advised to either perform DP donor typing to establish VXM compatibility, or to perform a FXM prospectively. Fortunately, many transplant centers have already begun HLA-DP typing (30) and the recent decision by the UNOS Board of Directors to extend required donor HLA typing to include DQA and DPB should make incomplete donor HLA typing much less common in the US (31). VXM has an exceptionally safe track record thus far. Still, we performed retrospective FXMs in the majority of the VXM only patients the results of which were generally available shortly after revascularization or

within hours of the surgery. Nonetheless, it may be desirable to disclose to patients the very small chance that foregoing a pretransplant FXM increases the risk of acute or hyperacute rejection if there were to be a lab or clerical error of the HLA typing.

As our results suggest, VXM is predictive of transplant compatibility comparable to cell-based assays. The benefit of VXM to reduce CIT for imported organs from certain locations may make VXM the preferred method to determine donor and recipient HLA compatibility, thus allowing organs to be shared across an extended geographic range such as in the US or Europe. This may be particularly important for the pancreas allograft, which is highly sensitive to ischemia-reperfusion injury. Additionally, by eliminating the FXM, pancreata can potentially be accepted from a geographic area that is farther than what is currently feasible. Practically speaking, organs harvested in the evening or at night that may have previously endured too much CIT while waiting to be transported on the first commercial flight in the morning may now be able to reach the transplant center with an acceptable CIT, rather than being discarded.

By using VXM only we have shown a significant reduction in CIT from certain UNOS regions, but not others. This is likely because of the low numbers of imported pancreata from each UNOS region, which makes it difficult to have adequate statistical power to compare transplants in specific UNOS regions. In spite of this, we did find that pancreata imported from UNOS regions 3 and 4 that proceeded to transplant with only a VXM had an average of 5.1 hours less CIT. Interestingly, all of the pancreata imported from east coast UNOS regions in this series proceeded to transplant with only a VXM. In other words, we were not able to expedite transplants using pancreata from east coast UNOS regions while still performing a pretransplant FXM. Data we obtained comparing the delay time from organ arrival to OR start time also supports the concept of expedited transplants in the setting of proceeding with only a pretransplant VXM. Clearly, the additional CIT necessary for performing a FXM is unlikely to be the sole reason for not importing pancreata for transplantation; yet, the timing of organ recoveries and available commercial airline transportation combined with a need for performing FXM surely impacted the feasibility of importing pancreata from these regions in some cases. Based on these data, it is apparent that the ability to minimize CIT by eliminating a FXM is a factor that can determine utilization.

Furthermore, while not the focus of this study there is a potential cost saving benefit of VXM compared to FCM. With VXM there is less personnel time and lower reagent costs, which has been associated with overall cost savings when applied to kidney transplantation (7). In the era of more cost conscious care, this may prove to be an important consideration for many transplant centers.

We acknowledge certain shortcomings in our analysis. First the analysis is limited by its retrospective nature, which is inherently subject to a selection bias, even if unintended. For example, organs that would have been expected to have a 20 hour CIT may have been rejected if an FXM was needed, since the FXM would push the CIT to 24 hours, the upper bound of what many centers would consider safe. However that same organ may have been accepted if it was known that transplant would proceed as soon as the organ arrived. On the

other hand, a pancreas that arrived with just 8 hours of CIT would have had the luxury of time, making it more likely to have both a VXM and FXM if deemed necessary. Regardless, the validity of VXM methods and cutoff values has been previously reported (12,13,32). This study is also limited by its relatively small sample size. Additional subjects in the both imported groups would have improved the study power.

In conclusion, we find in our single center retrospective study that VXM enables pancreas transplants to proceed safely without waiting for a FXM. For imported pancreata, CIT can be minimized without increasing risk of rejection or adversely affecting graft or patient survival. We believe the decision to supplant FXM with VXM is appropriate for all pancreas transplant candidates where complete donor typing has been obtained. Based on these data, broad implementation and standardization across transplant centers of VXM procedures including recipient HLA antibody testing and complete donor HLA typing could increase utilization of pancreas grafts by reducing CIT which in turn could improve outcomes by reducing reperfusion injury.

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Abbreviations

ACR	acute cellular rejection
AMR	acute antibody-mediated rejection
BMI	body mass index
cPRA	calculated panel reactive antibody
DM	diabetes mellitus
DSA	donor specific antibody
FXM	flow crossmatch
PRA	panel reactive antibody
SAB	single antigen bead
SP	solitary pancreas
SPK	simultaneous pancreas kidney
VXM	virtual crossmatch

References

1. Sollinger HW, Odorico JS, Becker YT, D'Alessandro AM, Pirsch JD. One thousand simultaneous pancreas-kidney transplants at a single center with 22-year follow-up. *Ann Surg.* 2009; 250(4):618–630. [PubMed: 19730242]

2. Ojo AO, Meier-Kriesche HU, Hanson JA, et al. The impact of simultaneous pancreas-kidney transplantation on long-term patient survival. *Transplantation*. 2001; 71(1):82–90. [PubMed: 11211201]
3. Speight J, Reaney MD, Woodcock AJ, Smith RM, Shaw JA. Patient-reported outcomes following islet cell or pancreas transplantation (alone or after kidney) in Type 1 diabetes: a systematic review. *Diabet Med*. 2010; 27(7):812–822. [PubMed: 20636963]
4. van Dellen D, Worthington J, Mitu-Pretorian OM, et al. Mortality in diabetes: pancreas transplantation is associated with significant survival benefit. *Nephrol Dial Transplant*. 2013; 28(5): 1315–1322. [PubMed: 23512107]
5. Gruessner RW, Gruessner AC. The current state of pancreas transplantation. *Nat Rev Endocrinol*. 2013; 9(9):555–562. [PubMed: 23897173]
6. Amico P, Honger G, Steiger J, Schaub S. Utility of the virtual crossmatch in solid organ transplantation. *Curr Opin Organ Transplant*. 2009; 14(6):656–661. [PubMed: 19741534]
7. Bingaman AW, Murphey CL, Palma-Vargas J, Wright F. A virtual crossmatch protocol significantly increases access of highly sensitized patients to deceased donor kidney transplantation. *Transplantation*. 2008; 86(12):1864–1868. [PubMed: 19104435]
8. Maglione M, Ploeg RJ, Friend PJ. Donor risk factors, retrieval technique, preservation and ischemia/reperfusion injury in pancreas transplantation. *Curr Opin Organ Transplant*. 2013; 18(1):83–88. [PubMed: 23254698]
9. Finger EB, Radoseovich DM, Dunn TB, et al. A Composite Risk Model for Predicting Technical Failure in Pancreas Transplantation. *American Journal of Transplantation*. 2013; 13(7):1840–1849. [PubMed: 23711225]
10. Axelrod DA, Sung RS, Meyer KH, Wolfe RA, Kaufman DB. Systematic evaluation of pancreas allograft quality, outcomes and geographic variation in utilization. *Am J Transplant*. 2010; 10(4): 837–845. [PubMed: 20121753]
11. Kandaswamy R, Stock PG, Skeans MA, et al. OPTN/SRTR 2011 Annual Data Report: pancreas. *Am J Transplant*. 2013; 13(Suppl 1):47–72. [PubMed: 23237696]
12. Ellis TM. Interpretation of HLA single antigen bead assays. *Transplant Rev (Orlando)*. 2013; 27(4):108–111. [PubMed: 23958238]
13. Ellis TM, Schiller JJ, Roza AM, Cronin DC, Shames BD, Johnson CP. Diagnostic accuracy of solid phase HLA antibody assays for prediction of crossmatch strength. *Hum Immunol*. 2012; 73(7):706–710. [PubMed: 22537747]
14. Baxter-Lowe LA, Cecka M, Kamoun M, Sinacore J, Melcher ML. Center-defined unacceptable HLA antigens facilitate transplants for sensitized patients in a multi-center kidney exchange program. *Am J Transplant*. 2014; 14(7):1592–1598. [PubMed: 24934640]
15. Cecka JM, Kucheryavaya AY, Reinsmoen NL, Leffell MS. Calculated PRA: initial results show benefits for sensitized patients and a reduction in positive crossmatches. *Am J Transplant*. 2011; 11(4):719–724. [PubMed: 21114658]
16. Yanagida R, Czer LS, Reinsmoen NL, et al. Impact of virtual cross match on waiting times for heart transplantation. *Ann Thorac Surg*. 2011; 92(6):2104–2110. discussion 2111. [PubMed: 22115224]
17. Park H, Lim YM, Han BY, Hyun J, Song EY, Park MH. Frequent false-positive reactions in pronase-treated T-cell flow cytometric cross-match tests. *Transplant Proc*. 2012; 44(1):87–90. [PubMed: 22310587]
18. Bray RA, Nickerson PW, Kerman RH, Gebel HM. Evolution of HLA antibody detection: technology emulating biology. *Immunol Res*. 2004; 29(1–3):41–54. [PubMed: 15181269]
19. Ogura K, Terasaki PI, Johnson C, et al. The significance of a positive flow cytometry crossmatch test in primary kidney transplantation. *Transplantation*. 1993; 56(2):294–298. [PubMed: 8356583]
20. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med*. 1969; 280(14):735–739. [PubMed: 4886455]
21. Karpinski M, Rush D, Jeffery J, et al. Flow cytometric crossmatching in primary renal transplant recipients with a negative anti-human globulin enhanced cytotoxicity crossmatch. *J Am Soc Nephrol*. 2001; 12(12):2807–2814. [PubMed: 11729251]

22. Duquesnoy RJ, Kamoun M, Baxter-Lowe LA, et al. Should HLA mismatch acceptability for sensitized transplant candidates be determined at the high-resolution rather than the antigen level? *Am J Transplant.* 2015; 15(4):923–930. [PubMed: 25778447]
23. Tait BD, Susal C, Gebel HM, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation.* 2013; 95(1):19–47. [PubMed: 23238534]
24. Brokhof MM, Sollinger HW, Hager DR, et al. Antithymocyte globulin is associated with a lower incidence of de novo donor-specific antibodies in moderately sensitized renal transplant recipients. *Transplantation.* 2014; 97(6):612–617. [PubMed: 24531846]
25. Niederhaus SV, Muth B, Lorentzen DF, et al. Luminex-based desensitization protocols: the University of Wisconsin initial experience. *Transplantation.* 2011; 92(1):12–17. [PubMed: 21512428]
26. Hahn, AB.; Land, GA.; Strothman, RM. Immunogenetics ASfHa. ASHI laboratory manual. 4. American Society for Histocompatibility and Immunogenetics; 2000.
27. Drachenberg CB, Torrealba JR, Nankivell BJ, et al. Guidelines for the diagnosis of antibody-mediated rejection in pancreas allografts—updated Banff grading schema. *Am J Transplant.* 2011; 11(9):1792–1802. [PubMed: 21812920]
28. Niederhaus SV, Levenson GE, Lorentzen DF, et al. Acute cellular and antibody-mediated rejection of the pancreas allograft: incidence, risk factors and outcomes. *Am J Transplant.* 2013; 13(11):2945–2955. [PubMed: 24102905]
29. Gruessner AC, Sutherland DE. Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. *Clin Transplant.* 2005; 19(4):433–455. [PubMed: 16008587]
30. Bray RA, Gebel HM. The New Kidney Allocation System (KAS) and the Highly Sensitized Patient: Expect the Unexpected. *Am J Transplant.* 2014; 14(12):2917. [PubMed: 25274464]
31. Tyan, D.; Bray, R. OPTN/UNOS Histocompatibility Committee Report to the Board of Directors. Jun 1–2. 2015
32. Middleton D, Jones J, Lowe D. Nothing's perfect: the art of defining HLA-specific antibodies. *Transpl Immunol.* 2014; 30(4):115–121. [PubMed: 24582684]

Figure 1.

Study design. Of the 153 patients that received simultaneous pancreas-kidney (SPK) transplants and solitary pancreas transplants 51 were imported from outside of our donor network. Of those, 39 proceeded to transplant with just a virtual crossmatch (VXM) while the 12 were not begun until a flow crossmatch (FXM) was performed and found to be negative.

*VXM only transplants had a flow crossmatch performed retrospectively

Figure 2.

Death-censored graft survival comparing imported VXM only vs. imported VXM + FXM vs. local VXM + FXM groups. Death-censored graft survival did not differ between groups at 1 year or at the terminal point in the study ($p = 0.45$).

Figure 3.

Cold ischemia times (CIT) of transplanted pancreata. Local pancreata had a shorter CIT than either of the imported groups (imported VXM only, 16.0 h vs. imported VXM + FXM, 17.5 h vs. local, 13.2 h, $p < 0.001$).

Figure 4.

Cold ischemia times (CIT) of transplanted pancreata originating from geographically similar UNOS regions. Pancreata recovered in regions 7,8,10 had similar CIT regardless of whether a FXM was performed prospectively or not (imported VXM only: 14.6 h (n=14) vs. imported VXM + FXM: 13.6 h (n=4) and CIT was similar to that of pancreata transplanted from our donor service area (13.2 h [n=101]) (p = 0.43). Pancreata recovered from regions 5 and 6 exhibited slightly numerically shorter CIT if the transplant proceeded forgoing a prospective FXM imported VXM only: 17.5 h (n=10) vs. imported VXM + FXM: 18.7 h (n=5), but the reduction in CIT was not statistically significant (p=0.9). Pancreata in regions 3 and 4 that were transplanted after only a VXM (n=9) had 5.1 hours less CIT than pancreata transplanted after waiting for a FXM (n=3) (95% CI [3.25, 6.98]) (p = 0.0001). Pancreata from regions 1, 2, 9, 11 averaged 17.5 hours of CIT and were only accepted if the prospective FXM could be waived (n=6). Source for UNOS map: www.unos.org/docs.UNOS_FactsFigure.pdf, page 9.

Figure 5.

Delay time of imported pancreata from organ arrival at hospital to patient arrival in the OR. Organs that went to transplant without waiting for a FXM spent 163 minutes less time on ice on average (imported VXM only: 149.9 min. vs. imported VXM+FXM: 296.3 min., $p < 0.001$)

Table 1

Donor Demographics

Abbreviations: DCD, donation after cardiac death; DBD, donation after brain death; HTN, hypertension

Donor Demographics					
	Imported		Local		
Pre transplant XM	VXM only	VXM + FXM	VXM + FXM	All	P
Number of Donors	39	12	102		
Mean Age (SD)	25.7 (10.9)	28.3 (13.5)	31.0 (12.4)	29.4 (12.3)	0.065
Gender, n (%)					
Male	20 (51.3)	7 (58.3)	64 (62.7)	91 (59.5)	
Female	19 (48.7)	5 (41.7)	38 (37.3)	62 (40.5)	0.46
Race, n (%)					
Caucasian	28 (71.8)	10 (83.3)	94 (92.2)	132 (86.3)	
Black	7 (17.9)	1 (8.3)	4 (3.9)	12 (7.8)	
Hispanic	4 (10.3)	0 (0)	3 (2.9)	7 (4.6)	
Asian	0 (0)	1 (8.3)	1 (1.0)	2 (1.3)	0.008
Type of Death, n (%)					
DCD	6 (15.4)	0 (0)	17 (16.7)	23 (15.0)	
DBD	33 (84.6)	12 (100)	85 (83.3)	130 (85.0)	0.29
Donor Criteria, n (%)					
Standard	39 (100)	12 (100)	99 (97.1)	150 (98.0)	
Expanded	0 (0)	0 (0)	3 (2.9)	3 (2.0)	0.47
Cause of Death, n (%)					
Anoxia	18 (46.2)	3 (25.0)	24 (23.5)	45 (29.4)	
Cerebrovascular/Stroke	5 (12.8)	4 (33.3)	21 (20.6)	30 (19.6)	
Head Trauma	13 (33.3)	5 (41.7)	50 (49.0)	68 (44.4)	
Other	3 (7.7)	0 (0)	6 (5.9)	9 (5.9)	0.29
History of Alcohol Abuse, n (%)					
Yes	7 (17.9)	1 (8.3)	8 (7.8)	16 (10.5)	
No	32 (82.1)	11 (91.7)	88 (86.3)	131 (85.6)	
Unknown	0 (0)	0 (0)	6 (5.9)	6 (3.9)	0.43
History of HTN, n (%)					
Yes	2 (5.1)	0 (0)	11 (10.8)	13 (8.5)	
No	37 (94.9)	12 (100)	91 (89.2)	140 (91.5)	0.57
Donor Blood Type, n (%)					
A	16 (41.0)	3 (25.0)	40 (39.2)	59 (38.6)	
AB	1 (2.6)	0 (0)	3 (2.9)	4 (2.6)	
B	3 (7.7)	2 (16.7)	10 (9.9)	15 (9.8)	
O	19 (48.7)	7 (58.3)	49 (48.0)	75 (49.0)	0.92

Table 2

Recipient Demographics

Abbreviations: BMI, Body Mass Index; DSA, Donor Specific Antibody; DM, Diabetes Mellitus

Recipient Demographics					
Pre transplant XM	Imported		Local		P
	VXM only	VXM + FXM	VXM + FXM	All	
Number of Patients	39	12	102	153	
Mean Age, yr (SD)	45.8 (9.0)	47.6 (10.4)	44.2 (8.7)	44.9 (8.0)	0.36
BMI, kg/m2 (SD)	26.0 (3.4)	24.9 (3.6)	25.4 (4.0)	25.5 (3.8)	0.61
Gender					
Male (%)	22 (56.4)	5 (41.7)	44 (43.1)	71 (46.4)	
Female (%)	17 (43.6)	7 (58.3)	58 (56.9)	82 (53.6)	0.35
Race, n (%)					
Asian	0 (0)	1 (8.3)	2 (2.0)	3 (2.0)	
Black	0 (0)	0 (0)	9 (8.8)	9 (5.9)	
Hispanic or Latino	0 (0)	0 (0)	5 (4.9)	5 (3.3)	0.087
White (Non Hispanic/Latino)	39 (100)	11 (91.7)	86 (84.3)	136 (88.9)	0.275
Blood type, n (%)					
A	15 (38.5)	5 (41.7)	37 (36.3)	57 (37.3)	
AB	2 (5.1)	0 (0)	10 (9.8)	12 (7.8)	
B	5 (12.8)	2 (16.7)	9 (8.8)	16 (10.5)	
O	15 (43.6)	5 (41.7)	46 (45.1)	68 (44.4)	0.83
Panel Reactive Antibody (PRA)					
Mean % Class 1 PRA, (SD)	18.6 (30.4)	22.7 (29.8)	3.8 (13.5)	9 (21.8)	<0.001
Mean % Class 2 PRA (SD)	14.2 (26.0)	26.1 (40.0)	2.5 (12.3)	7.3 (21.0)	<0.001
PRA >0% n,(%)	21 (53.8)	9 (75.0)	17 (16.7)	47 (30.7)	<0.001
PRA >20% n,(%)	15 (38.5)	6 (50.0)	11 (10.8)	32 (20.9)	<0.001
DSA Positive, n (%)	16 (41.0)	8 (66.7)	7 (6.9)	31 (20.3)	<0.001
Transplant Type, n (%)					
SPK	5 (12.8)	0, (0)	89 (87.3)	94 (61.4)	
SP	34 (87.2)	12 (100)	13 (12.7)	59 (38.6)	<0.001
Number of Previous Transplants, n (%)					
0	28 (71.8)	6 (50.0)	93 (91.2)	127 (83.0)	
1	9 (23.1)	4 (33.3)	7 (6.9)	20 (13.1)	
2	2 (5.1)	1 (8.3)	0 (0)	3 (2.0)	
3	0 (0)	1 (8.3)	2 (2.0)	3 (2.0)	0.001
Primary Native Disease, n (%)					
DM Type 1	37 (94.9)	12 (100)	81 (79.4)	140 (91.5)	
DM Type 2	0 (0)	0 (0)	9 (8.8)	9 (5.9)	
Other	2 (5.1)	0 (0)	2 (2.0)	3 (2.0)	0.243
Duration of Diabetes, yr (SD)	28.2 (10.9)	32.1 (11.4)	28.9 (9.0)	29.0 (9.8)	0.48
Missing, n (%)	0 (0)	0 (0)	33 (32.4)	33 (21.6)	

Recipient Demographics					
Pre transplant XM	Imported		Local	All	P
	VXM only	VXM + FXM	VXM + FXM		
Induction Immunosuppression, n (%)					
Depleting	20 (51.3)	8 (66.7)	28 (27.5)	56 (36.6)	0.003
Nondepleting	19 (48.7)	4 (33.3)	74 (72.5)	97 (63.4)	

Table 3

Biopsy-proven rejection rates at 90 days posttransplant and the terminal point of the study in simultaneous pancreas kidney (SPK) and solitary pancreas (SP) transplants. Comparisons were made between groups across all pancreas transplants, then again between like-transplants. Rates were low and were not significantly different between groups.

Rejection Rates				
	Imported		Local	
	VXM only	VXM + FXM	VXM + FXM	P
Rejection of both SP and SPK transplants 90 Days				
Free of Any	89.7	100	96	0.25
Free of ACR	89.7	100	96	0.25
Free of AMR	97.4	100	97.1	0.84
Free of Mixed	100	100	97.1	0.47
At Study Termination				
Free of Any	84.6	100	95.1	0.075
Free of ACR	84.6	100	95.1	0.075
Free of AMR	97.4	100	97.1	0.84
Free of Mixed	100	100	97.1	0.47
Rejection in SPK transplants 90 Days				
Free of Any	100	-	96.6	0.69
Free of ACR	100	-	96.6	0.69
Free of AMR	100	-	97.8	0.75
Free of Mixed	100	-	97.8	0.75
At Study Termination				
Free of Any	100	-	95.1	0.65
Free of ACR	100	-	95.1	0.65
Free of AMR	100	-	97.8	0.75
Free of Mixed	100	-	97.8	0.75
Rejection in SP transplants 90 Days				
Free of Any	88.2	100	92.3	0.51
Free of ACR	88.2	100	92.3	0.51
Free of AMR	97.1	100	92.3	0.55
Free of Mixed	100	100	92.3	0.18
At Study Termination				
Free of Any	82.3	100	92.3	0.31
Free of ACR	82.3	100	92.3	0.31
Free of AMR	97.1	100	92.3	0.55
Free of Mixed	100	100	92.3	0.18