Virtual Crossmatching in Kidney Transplantation: The Wait Is Over

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Disclosure Information: Dr Taber's institution receives research funding from Veloxis and Astellas.

Presented at the Southern Surgical Association 131th Annual Meeting, Hot Springs, VA, December 2019.

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Short title: Virtual Crossmatch in Kidney Transplantation

Background: Flow cytometric crossmatching (FCXM) is currently the method of choice for most transplant programs prior to kidney transplantation. In July of 2017, our program implemented the virtual cross-match, without a prospective physical cross-match, for the majority patients in the setting of a new kidney allocation system implemented by UNOS.

Study Design: A retrospective review was conducted to determine if virtual cross-matching could reduce cold ischemia time (CIT). Secondary outcomes included the incidence of delayed graft function and 1-year patient and allograft failure

Results: A total of 825 patients received a kidney transplant between 12/1/2014 and 7/1/2018, 505 in the pre-implementation group and 227 in the post-implementation group The CIT decreased between the pre-implementation era to post implementation era from 16.67±8.7 hrs. to 14.5±8.2 hrs. (p=0.002). On univariate analysis, DGF rates were similar between the two eras (19% Vs 17% p=0.415), despite having more DCD and higher KDPI donors in the post-implementation era. There was no difference in biopsy proven acute rejection rates were 28 (5.6%) vs 8 (3.5%) p=0.226, one-year graft loss (4% vs 3%, p=0.304) or patient death (2% vs 1%, p=0.567) between groups. On multivariable modeling for mean CIT and incidence of DGF, patients transplanted in the post-implementation era had an adjusted reduction in CIT of an estimated 2.35 hours (95% CI 1.15 to 3.55, p<0.001). Patients in the post-implementation era also had 26% lower odds of developing DGF (OR 0.74, 95% CI 0.48 to 1.14, p=0.170), after adjusting for covariates.

Conclusion: Kidney transplantation can be safely performed with virtual cross-matching, without a prospective physical crossmatch with improved CIT and potentially reduced DGF rates without increased risk of rejection.

INTRODUCTION

Prior to kidney transplant, donor-recipient compatibility is currently assessed by physical crossmatching at most programs. This is done by either cytotoxic assays or, more commonly, by flow cytometry. Physical crossmatching is costly, resource intensive and time consuming. The introduction of solid phase-based methods for detecting anti-HLA antibodies has been a significant technical advance that has increased the sensitivity and specificity of detecting antibodies directed against HLA class I and class II antigens (1, 2). With the increased use of solid phase assays, compatibility between donor and recipients can be assessed without requiring viable donor cells, relying on complete HLA typing of the donor and current antibody assessment of the recipient.(3,4,5,6) This technique is termed virtual crossmatching (VXM), which can be performed quickly and allows for timelier organ allocation decisions. Studies have shown that VXM allows transplantation access to highly sensitized patients. (3, 7) The safety and utility of VXM is well described and increasing number of centers are adopting this methodology. (3, 8, 9, 10, 11)

In July of 2017, our program implemented the VXM, allowing recipient to undergo transplantation without a prospective physical crossmatch for the majority patients in the setting of the new kidney allocation system (KAS) implemented by UNOS. The aim of this process improvement endeavor was to determine the impact of programmatic implementation of VXN on kidney transplant outcomes.

METHODS:

Study Design

This was a longitudinal retrospective review, using center specific UNOS Standard Transplant Analysis and Research (STAR) files linked with data acquired from the electronic health records (EHR, Epic) and manual chart abstraction. Patient were divided into two groups based on when they received their transplant. The pre-implementation era was from 12/1/2014 to 6/30/2017 and post-implementation era occurred between 7/1/2017 to 6/30/2018. Clinical follow-up for all patients was for a year post-transplant, or until death or graft loss.

Patients

Patients were included in this analysis if they were adult (>18 years of age at the time of transplant) kidney transplant recipients. Patients were excluded if they received a kidney transplant outside the prespecified time periods listed above or if they received a non-renal transplant, either concurrently or prior to the kidney transplant, including pancreas, liver, and heart or lung transplant recipients.

HLA Crossmatch Methodology

Predicted result final crossmatch results were based on HLA interpretation of DSA MFI values. Generally, a single DSA with MFI < 1,000 is considered negative. Consideration is given to MFI values that might be understated due to dilution across multiple epitopes in the SAB assay bead set. A positive VXM was defined as the presence or possible presence of DSA, the latter referring to cases where the presence or absence of DSA could not be established based on epitope reactivity patterns, MFI, assay background, and patient allosensitization history. HLA allele-specific antibodies were considered in the VXM whenever the level of resolution of HLA alleles and antibodies allowed. Historically positive donor-directed HLA antibodies (but

currently negative) were not considered to constitute a positive VXM. See Figure 1 for the VXM algorithm followed for this analysis.

Outcomes

The primary outcome for this analysis was to assess the impact of the implementation of the VXM on the mean cold ischemic times (CIT, in hours) compared between patients pre and post-implementation. CIT was also assessed for local and well as imported organs. Additional outcomes included the incidence of delayed graft function (DGF), defined as the need for at least one dialysis session within seven days of transplant, acute rejection rates, graft failure and death within one year of transplant. Rates of acute rejection were assessed in those that were deemed sensitized (calculated panel reactive antibody [cPRA} .20%) highly sensitized (cPRA >80%) and very highly sensitized (cPRA >98%).

Covariates

We analyzed for differences in patient demographics and transplant characteristics between the pre and post-implementation era to assess and adjust for potential confounding. Patient demographics that were assessed included age, sex, and race/ethnicity. Transplant characteristics included HLA mismatches, cPRA and previous kidney transplant recipients. Donor characteristics included age, race/ethnicity, kidney donor profile index (KDPI), cardiac death donors and import organs. Primary outcome was to determine if virtual cross-matching could reduce cold ischemia time (CIT). Secondary outcomes included the overall incidence of delayed graft function, defined as the need for dialysis within the first 7 days post-transplant, Rejection rates, number of daytime transplant, 1-year allograft failure and patient death within 1 year.

Patients transplanted prior to changes in allocation (12/2014), patient with <1-year follow-up, multiorgan and pediatric recipients were excluded from the study.

Statistical Analysis

Descriptive statistics are compared using means for continuous normally distributed data, medians for ordinal and continuous non-normally distributed data and percentages for proportions. Univariate comparative analysis and inferences were made using the Student's t-test, Mann Whitney U test, Fisher's exact test or the chi square test, as appropriate. Multivariable modelling, which adjusted for baseline variables that differed between groups or are known to influence the outcome of interest, was conducted using linear and logistic regression. Model assumptions were assessed for prior to final model analysis. All statistical analyses were conducted using SPSS v25.0 (IBM Corp, Armonk, NY).

RESULTS:

Baseline Characteristics

A total of 825 patients received a kidney transplant between 12/1/2014 and 7/1/2018; 24 patients were excluded for being <18 years of age at the time of transplant and 69 were excluded for being non-renal transplant recipients, leaving 732 patients in the final analysis. This included 505 in the pre-implementation era group and 227 in the post-implementation era group. See Figure 2 for the Consort diagram. In terms of baseline demographics, age, sex, race/ethnicity and incidence of diabetes were comparable between groups. With regards to transplant characteristics, HLA mismatches and cPRA were comparable between the groups. There was a numerically higher incidence of previous transplant recipients in the pre-implementation group (9.9% vs 5.7% p=0.063), which did not reach statistically significant difference. In terms of

donor characteristics, post-implementation era donors were significantly older (36±15 yrs vs 39±14 yrs, p=0.003) and more likely to have donation after cardiac death (DCD, 5.7% vs 15.4%, p<0.001). Finally, KDPI was higher in the post-implementation era group, as compared to the pre-implementation group (44 vs 35, p=0.028). See Table 1 for baseline comparisons between groups.

Virtual Crossmatch Implementation

Following the VXM implementation, the vast majority of physical crossmatches were performed retrospectively or concurrent with surgery. In 7 patients of the 227 cases (3%) conducted after the VXM implementation, a prospective crossmatch was requested (2 cases with donor offers that had HLA alleles that are not represented on the single antigens beads panel, 3 cases with allele specific antibodies and the exact donor allele could not be excluded from the originating lab typing, and 2 cases with high non-specific background reactivity). The retrospective crossmatch correlated with the physical crossmatch in all cases with the exception of those that had positive autologous crossmatch or false positive crossmatches due to drug interference from antibody therapies such as rituximab and IVIG.

Outcomes

After implementation of the VXM policy, there was a clinically and statistically significant decrease in the mean CITs. The CIT decreased between the pre-implementation era to post implementation era from 16.67±8.7 hrs. to 14.5±8.2 hrs. (p=0.002). This difference in CIT was significant for both local (14.6±8.0 hrs vs 12.3±7.1 hrs, p=0.002) and import organs (24.5±5.5hrs vs 22.1±1.6, p=0.011). On univariate analysis, DGF rates were similar between the two eras (19% Vs 17% p=0.415), despite having more DCD and higher KDPI donors in the post-

implementation era. There was no difference in one year graft loss (4% vs 3%, p=0.304) or patient death (2% vs 1%, p=0.567) between groups. See Table 2 for outcome comparisons between groups. There were no cases of hyperacute rejection during this period. Biopsy proven acute rejection rates were 28 (5.6%) vs 8 (3.5%) p=0.226 in the pre and post implementation era (see Table 2 and Figure 3).

Due to differences in baseline characteristics, we performed multivariable modeling for the outcomes of mean CIT (linear regression) and incidence of DGF (logistic regression). Both models were adjusted for recipient age, race/ethnicity, history of diabetes, and donor DCD and KDPI. For the outcome of CIT, patients transplanted in the post-implementation era had an adjusted reduction in CIT of an estimated 2.35 hours (95% CI 1.15 to 3.55, p<0.001). Patients in the post-implementation era also had 26% lower odds of developing DGF (OR 0.74, 95% CI 0.48 to 1.14, p=0.170), after adjusting for covariates.

DISCUSSION:

Since the introduction of the cytotoxic crossmatch by Terasaki and Patel in 1969, physical crossmatching has been the cornerstone technique for preventing hyperacute antibody mediated rejection. (12) Even with the refinement in methods and advent of flow cytometric crossmatch, there is still a need for access for donor lymphocytes cells to perform the physical crossmatch. This inevitably leads to delays in suitable organ allocation, resulting in increased cold ischemia times. (13) Within the contemporary KAS, where substantial priority points are given to those with very high cPRAs, this issue is even more pronounced. Further, KAS implementation requires sharing of organs over longer distances and therefore any process which can make allocation more efficient and reduce cold ischemia times is of vital importance. (14)

The introduction of single antigen bead (SAB) testing is the principle driver behind the ability to use VXN testing to predict actual matching and to thereby avoid a prospective XM. By omission of prospective crossmatching using actual donor tissue, we were able decrease the CIT by more than 2 hours. This decrease is directly a result of not needing donor tissue to assess for donor-recipient matching. It is also important to note that the difference in CIT demonstrated was seen in both local and imported organs. Thus, the logistical issues with physical transporting donor tissue to the transplant center, even when the donor is considered local, is significant and leads to delays in assessment.

Our results demonstrated that the reduction in CIT allowed for similar rates of DGF even in the context of utilizing more marginal donors in the post-implementation era, as evidence by having significantly more DCDs and higher KDPIs. After adjustment for these differences, there was 26% lower odds of developing DGF in the post-implementation era, which did not reach statistical significance ($p=0.170$). Our results are consistent with previous studies assessing CIT and DGF. Shrestha et al looking at the logistical factors impacting cold ischemia times found virtual crossmatching decreased the cold ischemia times by 3hrs (13). Utilization of VXM is not just limited to the kidneys. Eby et al utilizing VXM for pancreas transplant were able to decrease the CIT by 3 hrs. there by increasing the ability to import and safely transplant pancreas. (15)

With Virtual crossmatching and SAB testing, we were able to safely transplant patients in an efficient manner, irrespective of cPRA. In our analysis, in the post-implementation era, more than 60% of the patients were sensitized with a cPRA >20%, while nearly one-third of patients had cPRA >80% and one in eight patients had cPRA >98%. Despite this, we had no hyperacute rejections and importantly, on retrospective crossmatch performed after completion of the

transplant, there was no discordance with the VXM results. Acute rejection rates were similar between the pre and post-implementation era groups and consistent with contemporary rates across the US. These results demonstrate we can safely move forward with the transplant using the VXM results, regardless of patient's sensitization.

These results, demonstrating the ability to predict the final crossmatch and concordance with the physical crossmatch is consistent with previous studies.(3,,8,16,17,18,19) Bray et al described the utilization of VXN and achieved similar outcomes in both low and highly sensitized patients, as compared to previous XM methods.(3) Turner et al published the Scottish experience and demonstrated that among 257 patients transplanted (42%) with VXM, none experienced a positive retrospective crossmatch and the authors found no difference in the rejection rates or long term graft survival. (8) Piazza et al published a study that demonstrated excellent correlation between VXM, physical crossmatch and flow cytometry crossmatch (97% and 90% respectively). Most importantly, in the Piazza study, the sensitivity of the VXM 100%; this high value was related to the lack of false-negative DSA results and is consistent with what we demonstrated in our analysis (19).

Since the advent of KAS, matched candidates with high (>98%) cPRA) are given priority over local candidates with lower cPRA. (20) Paramesh et al reported that the most common reason the intended recipient was not able to receive the allocated KAS organ was a positive crossmatch.(21) With the utilization VXM we were able to import organs over long distances and transplant them in the intended recipient safely and expeditiously, reducing CIT without any negative consequences on graft outcomes. Further, by implementing this protocol, we were also able to nearly eliminate the need to bring in back-up recipients to the hospital in case of a possible physical crossmatch in the intended primary recipient. This is particularly relevant in a

state like South Carolina the majority of patients liver long distances from the transplant center. Although not measured in this analysis, we expect that reducing the need to call in back-ups has a positive impact of those on the waiting list and could lead to decreased costs and anxiety for back up recipients.

An added advantage to the surgeon is the ability to get to the OR more electively, possibly decreasing the number of middle of the night transplants there by improving the quality of life and decreasing burnout in the long run.

The analysis has a number of limitations. It is a single center retrospective analysis. There were differences between the pre and post-implementation groups, including DCDs and KDPI, which clearly influence outcomes. We attempted to address this potential confounding through multivariable modeling, but there may be residual and unmeasured confounding that remains. Thus, these results should be taken in the context of a single center retrospective analysis. Also, the transplant population in South Carolina, which includes a high proportion of African-Americans and highly sensitized individuals may not be fully applicable to all transplant centers. It is important to note that these characteristics are considered high immunologic risk factors, and thus the low rejection rates and no hyperacute rejections should provide comfort to transplant surgeons that these results can be applied to lower risk populations. There are multiple other factors, including recipient travel time, organ transport logistics, need for dialysis pre- transplant and operation room availability the clearly impact CITs and cannot be mitigated through the use of VXM without the need for physical crossmatch prior to surgery.(13) Further, the success of the VXM depends on the organ procurement organization (OPO) to provide the complete HLA profile of the donor and the success of the program depends on the HLA expertise at the center and surgeons comfort level with the HLA director.

CONCLUSION:

Kidney transplantation can be safely performed without the need of a physical crossmatch in the vast majority of patients. When done by a well-trained and experienced HLA laboratory, coupled with direct communication with the OPO, the use of VXM as a final decision to proceed with transplant across transplant centers can lead to decreased CIT and potentially lower DGF rates without increased risk of rejection

REFERENCES:

1. Pei R, Lee JH, Shih NJ, et al. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. Transplantation. 2003; 75:43–49.
2. El-Awar N, Lee J, Terasaki PI. HLA antibody identification with single antigen beads compared to conventional methods. Hum Immunol. 2005; 66:989–997.
3. Bray RA, Nolen JD, Larsen C, et al. Transplanting the highly sensitized patient: The emory algorithm. Am J Transplant. 2006;6(10):2307-15.
4. 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.
5. Reed EF, Rao P, Zhang Z, et al. Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA. Am J Transplant. 2013;13 (7):1859-70.
6. Konvalinka A, Tinckam K. Utility of HLA Antibody Testing in Kidney Transplantation. Journal of the American Society of Nephrology : JASN. 2015;26(7):1489-502.
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):1864-8.
8. Turner D, Battle R, Akbarzad-Yousefi A, Little AM. The omission of the "wet" pre-transplant crossmatch in renal transplant centres in Scotland. Hla. 2019;94(1):3-10.

9. Taylor CJ, Kosmoliaptsis V, Sharples LD, et al. Ten-year experience of selective omission of the pretransplant crossmatch test in deceased donor kidney transplantation. Transplantation. 2010;89(2):185-93.
10. Bielmann D, Honger G, Lutz D, et al. Pretransplant risk assessment in renal allograft recipients using virtual crossmatching. Am J Transplant. 2007;7(3):626-32.
11. Worsley CM, Mayne ES, Suchard MS. Luminex-based virtual crossmatching for renal transplantation in South Africa. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde. 2011;102 (1):40-3.
12. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. The New England journal of medicine. 1969;280 (14):735-9.
13. Shrestha S, Bradbury L, Boal M, et al. Logistical Factors Influencing Cold Ischemia Times in Deceased Donor Kidney Transplants. Transplantation. 2016;100(2):422-8.
14. Lunz J, Hinsdale L, King C, et al. The coordination of allocation: Logistics of kidney organ allocation to highly sensitized patients. Human immunology. 2017;78(1):16-8.
15. Eby BC, Redfield RR, Ellis TM, et al. Virtual HLA Crossmatching as a Means to Safely Expedite Transplantation of Imported Pancreata. Transplantation. 2016;100(5):1103-10.
16. Morris GP, Phelan DL, Jendrisak MD, Mohanakumar T. Virtual crossmatch by identification of donor-specific anti-human leukocyte antigen antibodies by solid-phase immunoassay: a 30-month analysis in living donor kidney transplantation. Human immunology. 2010;71 (3):268-73.
17. Tambur AR, Ramon DS, Kaufman DB, et al. Perception versus reality?: Virtual crossmatch--how to overcome some of the technical and logistic limitations. Am J Transplant. 2009;9 (8):1886-93.

18. Sullivan HC, Dean CL, Liwski RS, et al. (F)Utility of the physical crossmatch for living donor evaluations in the age of the virtual crossmatch. Human immunology. 2018;79 (10):711-5.
19. Piazza A, Ozzella G, Poggi E, et al. Virtual crossmatch in kidney transplantation. Transplantation proceedings. 2014;46 (7):2195-8.
20. Friedewald JJ, Samana CJ, Kasiske BL, et al. The kidney allocation system. The Surgical clinics of North America. 2013;93(6):1395-406.
21. Paramesh AS, Neidlinger N, Salvatore M, et al. OPO Strategies to Prevent Unintended Use of Kidneys Exported for High PRA (>98% cPRA) Recipients. Am J Transplant. 2017;17(8):2139-43.

Table 1. Baseline Characteristics Compared Between the Pre- and Post-Implementation Patients

Baseline characteristic	**Pre-implementation (n=505)**	**Post-implementation (n=227)**	**p Value**
Age, y, mean±SD	52±14	53±13	0.300
Female sex, %	38.4	40.1	0.668
African American, %	59.0	59.9	0.818
Re-transplant, %	9.9	5.7	0.063
Diabetes, %	38.8	40.1	0.744
Human leukocyte antigen mismatch, median (IQR)	4 (3,5)	4 (3,5)	0.356
cPRA, median (IQR)	43 (0, 89)	46 (0, 89)	0.736
cPRA>20%, %	65.0	60.4	0.232
cPRA>80%, %	29.3	30.4	0.765
cPRA>98%, %	12.7	12.8	0.969
Donor age, y, mean±SD	36±15	39±14	0.003
Donor female, %	39.0	43.6	0.241
Donor African American, %	24.6	20.7	0.255
Donor after cardiac death, %	5.7	15.4	<0.001
Kidney Donor Profile Index, median (IQR)	35 (16, 60)	44 (27, 60)	0.028
Import organ, n (%)	109 (22)	54 (24)	0.503

cPRA, calculated panel reactive antibody

Table 2. Clinical Outcomes Compared Between the Pre- and Post-Implementation Patients

Outcome	**Pre-implementation (n=505)**	**Post-implementation (n=227)**	**p Value**
Cold ischemia time, h, mean±SD	16.67±8.7	14.6±8.03	0.002*
Local donor	14.5±8.2	12.3±7.1	0.002*
Import donor	24.5±5.5	22.1±6	0.011*
Delayed graft function, n (%)	98 (19)	38 (17)	0.413
Graft failure within 1 year, n (%)	22 (4)	6 (3)	0.304
Patient death within 1 year, n (%)	11 (2)	3 (1)	0.567
Biopsy proven acute rejection, n (%)	28 (5.6)	8 (3.5)	0.226
Either borderline or acute rejection, n (%)	41 (8.2)	14 (6.2)	0.327

*Statistically significant.

Figure Legend

Figure 1. Crossmatch algorithm detailing the algorithm that was followed for how potential donor and recipient pairs are assessed for compatibility using the VXM protocol. The 4 main decision points are labeled by the numbers 1 through 4. These points delineate when a virtual crossmatch is adequate to proceed with transplant or whether the offer should be declined. cPRA, calculated panel reactive antibody; DSA, donor specific antibody; FCXM, flow cytometric crossmatching; MFI, mean fluorescence intensity; Tx, transplant; VXM, virtual crossmatch

Figure 2. Consort diagram detailing how the pre and post cohorts were formed and the primary outcomes in each group. CIT, cold ischemia time; DGF, delayed graft function

Figure 3. Comparison of rejection-free survival rates in patients before and after implementation of the virtual crossmatch protocol.

Precis

The results of this analysis demonstrated that kidney transplantation can be safely performed with virtual crossmatching, without a prospective physical crossmatch, which leads to improved cold time, similar rates of acute rejection, and potentially reduced delayed graft function rate.