Human Immunology 78 (2017) 72–79

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Root cause analysis of limitations of virtual crossmatch for kidney allocation to highly-sensitized patients

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A R T I C L E I N F O

Article history:
Received 13 July 2016
Revised 11 November 2016
Accepted 14 November 2016
Available online 15 November 2016

Keywords:
Anti-HLA antibodies
Virtual crossmatch
Deceased donor kidney transplantation

A B S T R A C T

Efficient allocation of deceased donor organs depends upon effective prediction of immunologic compatibility based on donor HLA genotype and recipient alloantibody profile, referred to as virtual crossmatching (VCXM). VCXM has demonstrated utility in predicting compatibility, though there is reduced efficacy for patients highly sensitized against allogeneic HLA antigens. The recently revised deceased donor kidney allocation system (KAS) has increased transplantation for this group, but with an increased burden for histocompatibility testing and organ sharing. Given the limitations of VCXM, we hypothesized that increased organ offers for highly-sensitized patients could result in a concomitant increase in offers rejected due to unexpectedly positive crossmatch. Review of 645 crossmatches performed for deceased donor kidney transplantation at our center did not reveal a significant increase in positive crossmatches following KAS implementation. Positive crossmatches not predicted by VCXM were concentrated among highly-sensitized patients. Root cause analysis of VCXM failures identified technical limitations of anti-HLA antibody testing as the most significant contributor to VCXM error. Contributions of technical limitations including additive/synergistic antibody effects, prozone phenomenon, and antigens not represented in standard testing panels, were evaluated by retrospective testing. These data provide insight into the limitations of VCXM, particularly those affecting allocation of kidneys to highly-sensitized patients.

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1. Introduction

Implementation of solid-phase immunoassays (SPI) to detect anti-HLA antibodies [1,2] has revolutionized prediction of immunologic compatibility in organ transplantation. Traditionally, definitive evaluation of compatibility between patients and potential donors relies on cellular crossmatch testing (CXM) to detect antibodies against donor alloantigens [3,4]. Specificity in identifying anti-HLA antibodies afforded by SPI has enabled refinement of algorithms supporting organ allocation. Broad categorization of alloantigen sensitization by panel reactive antibody (PRA) was replaced with a calculated PRA (cPRA) determined by the specific anti-HLA antibodies present and the population frequencies of target HLA antigens [5]. This permits prediction of CXM results for potential donors with known HLA genotypes, or VCXM [6,7]. Implementation of specific anti-HLA antibody information and VCXM avoidance of donors against which patients have donor-specific antibodies (DSA) has decreased deceased donor kidney offers declined due to a positive CXM concurrent with an increase in organs transplanted to highly-sensitized (cPRA ⩾ 80%) patients [8,9].

To further reduce disadvantage due to sensitization against allogeneic HLA, a revised KAS prioritizing increased allocation of organs to the most highly-sensitized (cPRA 98–100%) patients was implemented in December 2014 [10]. This has increased access for the most highly sensitized patients, with 100% cPRA patients receiving 10.3% of deceased donor kidney transplants in the new KAS era, compared to only 1.0% previously [11,12]. Ensuring equitable transplant access is important, though it comes with

Abbreviations: cPRA, calculated panel reactive antibody; CXM, crossmatch; FCXM, flow cytometric crossmatch; KAS, revised kidney allocation system; MCS, mean channel shift; MFI, mean fluorescence intensity; PRA, panel reactive antibody; SPI, solid phase immunoassay; VCXM, virtual crossmatch.

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http://dx.doi.org/10.1016/j.humimm.2016.11.003
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the burden of increased transit time due to regional (increased 42%) and national (increased 50%) sharing, increased rates of delayed graft function, and a 10% increase in discard rates. These costs prompt careful evaluation of the effectiveness of the tools used.

The predictive ability of VCXM is highly dependent on SPI for identification of clinically-relevant alloantibodies. In clinical practice, VCXM correctly predicts CXM results in 89–97% of cases [9,13–19]. However, VCXM accuracy can be markedly reduced for highly-sensitized patients [16]. Compared to the overall low 0.4% national rate for deceased donor kidney offer declines due to positive CXM, 6.8% of offers to patients with cPRA 80–97% and 8.3% of offers to patients with cPRA ⩾ 98% were refused due to positive CXM [9]. A recent study suggested that as much as 16% of offers to highly-sensitized patients could result in positive CXM [19]. Identifying the limitations of VCXM for highly-sensitized patients is essential for the success in allocation of organs to these difficult to match patients.

VCXM limitations are well-recognized and include issues of incomplete donor HLA genotype information [14,17,19,20] and technical factors specific to SPI. Technical issues related to detection of anti-HLA antibodies by SPI include use of appropriate mean fluorescence intensity (MFI) cutoff values [21,22], additive effects of low-concentration DSA [13,22], false positive reactions against the microbeads or non-native HLA epitopes [23,24], and false negative SPI results due to inhibition by interfering substances or prozone effects [25–28]. We hypothesized that all of these factors may disproportionately affect very highly-sensitized patients and impair VCXM approaches relied upon to support the new KAS. Here, we retrospectively analyze VCXM and flow cytometric CXM (FCXM) performed for 645 deceased donor kidney offers at our institution before and after implementation of the revised KAS to identify causes underlying VCXM failures.

2. Materials and methods

2.1. Study population

Histocompatibility testing performed at the University of California San Diego Immunogenetics and Transplantation Laboratory for patients with end-stage renal disease being evaluated for kidney transplantation at UCSD or Rady Children's Hospital between December 2013 and December 2015 were included. Immunologic compatibility for patients presenting with a potential ABO-compatible living donor (n = 179) was tested by FCXM regardless of anti-HLA antibody profile. Patients registered for deceased donor kidney or kidney-pancreas transplantation had HLA serologic specificities against which the patient had clinically-relevant alloantibodies listed as avoid antigens in UNET. Immunologic compatibility for deceased donor kidney and kidney-pancreas transplantation was determined by prospective FCXM (n = 645). All laboratory testing was completed as part of standard of care.

2.2. Alloantibody testing

All patients were tested for anti-HLA antibodies by SPI as part of standard of care treatment. Patients were screened for anti-HLA antibodies by FlowPRA Class I and Class II bead-based flow cytometric assays (One Lambda) using FACSCanto or FACSCalibur instruments (BD). Anti-HLA antibodies detected by FlowPRA were identified using LABScreen Single Antigen HLA Class I and Class II bead assays using a Labscan 100 (Luminex). Normalized mean fluorescence intensity (MFI) values of 500 were used for the limit of detection for SAB. Normalized MFI values of 3000 were used to identify alloantibodies predicted to cause a positive CXM. Extended alloantibody testing was performed on neat sera, or sera diluted 1:10 in PBS or treated with EDTA (5 µl 6% EDTA solution added to 95 µl serum for samples with control bead MFI values out of range) [29], using LABScreen Single Antigen HLA Class I and Class II Supplements (One Lambda) and LABScreen MICA Single Antigen (One Lambda) assays on a Labscan 100. All data were analyzed using HLA Fusion software (One Lambda).

2.3. Cellular crossmatch testing

Donor lymphocytes isolated from peripheral blood, spleen, or lymph node samples by density gradient separation using RosetteSep Lymphocyte Enrichment kit (StemCell Technologies) were treated with 2 mg/ml pronase (Sigma) for 20 min at 37 °C. Donor cells were incubated in duplicate with current (typically <30 days old) and historical peak (maximum cPRA within the last 12 months) recipient serum for 20 min at room temperature. Cells were labeled with anti-CD3 PerCP (SK7, BD Biosciences) anti-CD19 PE (SJ25-C1, BD Biosciences), and goat F(ab')2 anti-human IgG FITC (Jackson) for 20 min at 4 °C, washed, and analyzed using a FACSCalibur or FACSCanto. Alloantibody binding was determined by calculating mean channel shift (MCS) of cells incubated with patient serum as compared to cells incubated with control normal human serum; MCS ⩾ 16 was considered positive for T cell FCXM and MCS ⩾ 32 was considered positive for B cell FCXM.

2.4. HLA typing

All patients and donors were typed for HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA, -DQB1, -DPA, and -DPB1. Any donors without complete typing available in UNET were re-typed in the UCSD ITL. Patient and donor typing prior to July 2015 were performed using LABType SSO (One Lambda) on a Labscan 100 and analyzed using HLA Fusion software. After July 2015, deceased donor typing was performed using the LinkSeq real-time pcr assay (Linkage Biosciences) on a Lightcycler 480 (Roche) and analyzed using SureTyper software (Linkage Biosciences). Ambiguities in HLA tying were resolved using MicroSSP Allele Specific trays (One Lambda).

2.5. Statistical analyses

Proportional data among groups were analyzed using Fisher's exact test. Sensitivity and specificity were calculated using receiver-operator curve analyses. Correlation of DSA MFI and FCXM MCS was performed by linear regression and analyzed by goodness of fit. All statistical analyses were performed using Prism 6 (Graph Pad).

3. Results

3.1. Revised KAS increased kidney offers to very highly-sensitized (cPRA ⩾ 98%) patients but did not result in statistically significant increases in positive FCXM overall

We evaluated the impact of the revised KAS by examining offers for deceased donor kidneys to patients at the kidney transplant programs at UCSD and Rady Children's Hospital San Diego. Policy at our centers is to list HLA antigens as unacceptable in UNET when the average normalized SPI bead MFI for a given antigen is >3000. This leverages UNET to perform VCXM (with the caveat of not accounting for information for HLA-DPB1, -DQA1, and -DPA1 prior to 1/21/2016, or alloantibodies not listed in UNET). Alloantibody profiles for patients on deceased donor crossmatch lists for the first 13 months of the revised KAS (12/4/2014–12/31/2015) were compared with patients receiving an offer (based on FCXM) for

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