Human Immunology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Human Immunology

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

Improving the performance of virtual crossmatch results by correlating with nationally-performed physical crossmatches: Obtaining additional value from proficiency testing activities

Sean M. Wrenn[a,*], Carlos E. Marroquin[a], Donna-Sue Hain[b], Sarah K. Harm[b], Jaime A. Pineda[a], Paulette B. Hammond[b], Diti H. Shah[b], Susan E. Hillyard[b,a], Mark K. Fung[b]

[a] University of Vermont Robert Larner, M.D. College of Medicine, Department of Transplant Surgery, Burlington, VT, United States
[b] University of Vermont Robert Larner, M.D. College of Medicine, Department of Pathology, Burlington, VT, United States

ARTICLE INFO

Keywords:
Blood grouping and crossmatching
Organ transplantation
Transplantation immunology
HLA antigens
Antibodies

ABSTRACT

Purpose: When donor specific HLA antibodies (DSA) are identified, the predictive value of whether a certain strength of reactivity (mean fluorescence intensity, MFI) leads to a positive crossmatch is uncertain. To determine this, we compared the DSA MFI results we generated locally for nationally distributed proficiency samples against the percentage of other laboratories reporting a positive crossmatch.

Method: DSA MFI from single antigen beads reported by our laboratory for nationally-distributed proficiency testing survey samples was compared against the aggregate percentage of participating laboratories reporting the crossmatch positive using direct, antiglobulin-enhanced microcytotoxic (CDC-AHG), or flow cytometric methods from 2011 to 2015.

Results: 180 surveys were analyzed. Positive CDC-AHG and flow cytometric crossmatches were associated with MFI greater than 8554 and 2748 respectively for HLA class I, and 6919 and 3707 respectively for class II. Institutional MFI less than 3000 had high positive predictive values (0.98, 0.85, 0.81) for negative direct, AHG, and flow crossmatches, while MFI greater than 8000 had high negative predictive values for a positive direct, AHG, and flow crossmatches (1.00, 1.00, 0.97).

Conclusion: Review of locally-generated MFI results as part of participating in proficiency testing allow for predictability of crossmatch results against other laboratories, providing a replicable model for other participating centers.

1. Introduction

A central tenet of kidney transplantation donor and recipient matching is that human leukocyte antigen (HLA) incompatibility frequently results in either acute or chronic immune-mediated allograft rejection and deleterious transplant outcomes [1]. It has been established for over 40 years that a positive preoperative cytotoxic crossmatch (XM) resulted in inferior graft survival following kidney transplant [2]. Alternative assays and methods have evolved over time that have proven even more sensitive than the classic direct cytotoxic XM. In particular, the development of detection methods utilizing microspheres coated with purified HLA molecules (Luminex technology) allows for a more sensitive method of detecting HLA antibodies, and greater accuracy of determining the donor-specific alloantibodies (DSA). With a report of the identified HLA antibodies using the Luminex method, a comparison against a potential donor's HLA type can be made in a "virtual" crossmatch to determine the likelihood of a positive crossmatch and likelihood of early allograft loss. However the clinical significance of low-level DSA that does not produce a positive XM is unclear [3,4]. It would be unknown whether the sample if tested in other laboratories might produce a positive XM result. Therefore, in this study we retrospectively reviewed reports from nationally

Abbreviations: DSA, Donor Specific Antibody/Donor Specific Allo-antibody; CAP, College of American Pathologists; AMR, antibody-mediated rejection; XM, Cross Match; FC, Flow Cytometry; SABA, Single Antigen Bead Assay; UA, Unacceptable Antigen; SD, Standard Deviation; MFI, mean fluorescent intensity; DNA, deoxyribonucleic acid; HLA, human leukocyte antigen; NIH, National Institutes of Health; AHG, anti-human globulin; PPV, Positive Predictive Value; NPV, Negative Predictive Value; UVM, University of Vermont; CDC, complement-dependent cytotoxicity; IgG, immunoglobulin G; DTT, dithiothreitol

* Corresponding author at: 111 Colchester Avenue, Burlington, VT 05401, United States.
E-mail addresses: Sean.Wrenn@uvmhealth.org (S.M. Wrenn), Carlos.Marroquin@uvmhealth.org (C.E. Marroquin), DonnaSue.Hain@uvmhealth.org (D.-S. Hain), Sarah.Harm@uvmhealth.org (S.K. Harm), Jaime.Pineda@uvmhealth.org (J.A. Pineda), Paulette.Hammond@uvmhealth.org (P.B. Hammond), Diti.Shah@uvmhealth.org (D.H. Shah), Susan.Hillyard@uvmhealth.org (S.E. Hillyard), Mark.Fung@uvmhealth.org (M.K. Fung).

https://doi.org/10.1016/j.humimm.2018.05.009
Received 22 February 2018; Received in revised form 30 May 2018; Accepted 31 May 2018

distributed samples for HLA antibody testing and crossmatching, and correlated our institutional HLA laboratory Luminex antibody results for strength of reactivity as MFI against the percentage of laboratories that were able to obtain a positive crossmatch by either the less sensitive cytotoxic method or the more sensitive flow cytometric method.

2. Materials and methods

The aggregate results from the performance of HLA antibody testing and crossmatching in a national proficiency testing program (2011–2015, College of American Pathologists (CAP) proficiency surveys) was reviewed. The aliquots of the same specimens are distributed nationally and tested in a blinded fashion by the major transplant institutions around the country on a routine basis as part of their accreditation requirements. Each survey involves a standard sample of lymphocytes, and a standard serum sample, which are then tested using the preferred modalities of each testing center. Crossmatch methods included direct complement-directed cytotoxicity (CDC), anti-human globulin (AHG) augmented, and flow cytometry. The protocols outlined in the next three paragraphs are the techniques utilized by our institution, but do not necessarily reflect the protocols used at the various participating CAP testing centers.

2.1. Procedure for T-cell crossmatch (AHG-augmented)

For each CAP T-Cell XM survey, 6 samples are received: 2 lymphocyte samples and 4 recalcified plasma (sera) samples. Sera were stored at 4 degrees celsius until use and tests were performed within 24 h of receiving each sample. The 4 CAP sera samples first undergo serial dilutions with RPMI media. Appropriate positive and negative controls were utilized and each test was performed in duplicate. Standard incubation times (30 min at room temperature) were utilized for cells and sera. After incubation and four wash steps (5 µl RPMI to each well, sliding the tray over the magnet and flicking), anti-human globulin (AHG, Goat IgG Anti-Human Kappa (Free and Bound Light Chains, 1 µL) is added (2 min incubation time). Rabbit complement (5 µL) is added to initiate cell lysis if antibody binding of the lymphocytes occurs. Following this, the cells, bound antibody, AHG, and complement incubate for 60 min. FluoroQuench is added which stops the complement-dependent cell lysis, staining the dead cells red and the live cells green. Lymphocyte samples, controls, and sera dilutions were added to a standard crossmatch tray. Testing was performed by laboratory medical technologists in the HLA laboratory. Lymphocyte samples are labeled as “Donor” and sera samples as “Recipient”. Crossmatch grading occurs via the NIH scoring system of 0–8, based on the percentage of stained dead cells viewed on microscopy (> 21% stained dead cells graded as positive XM).

2.2. Procedure for B-cell crossmatch (NIH) method

The B-Cell (Class II) Crossmatch protocol is similar to the T-Cell, however there are 4 total samples tested (2 B Lymphocyte samples, and 2 re-calcified plasma samples). Following preparation and appropriate serial dilution the 2 lymphocyte samples are crossed against the 2 sera samples, with appropriate positive and negative controls, on a crossmatch tray via our standard protocol. Cells and sera were incubated for 60 min at room temperature. 5 µL of Rabbit DR complement is added to each well, followed by a 60 min incubation. Unlike the T-Cell XM, no AHG is added (direct XM). Similar to the T-Cell crossmatch, the results are recorded on CAP Survey forms.

In each crossmatch protocol, IgM interference was mitigated by the use of heat inactivation. Heat inactivation was only performed when the auto or negative controls appeared positive.

Table 1
Stratification of sera by cumulative DSA and associated risk of positive direct, AHG, or flow crossmatch.

Cumulative MFI value of sample	Total number of samples (n)	Mean CAP % Direct XM Positive (SD)	Mean CAP % AHG XM Positive (SD)	Mean CAP % Flow XM Positive (SD)
0	43	2% (2.8)	3.3% (6.2)	8% (19.8)
1–3000	9	3.1% (2.0)	5.7% (5.1)	30.8% (28.5)
3001–8000	9	7.7% (6.5)	14.4% (15.0)	58.3% (19.8)
8001–15,000	26	34.7% (29.6)	60.7% (27.5)	93.9% (8.3)
15,001–20,000	48	84.75% (21.2)	91.9% (14.8)	99.5% (1.0)
20,001–30,000	27	85.5% (24.3)	93.6% (17.4)	99.7% (0.7)
30,001–40,000	11	93.6% (7.5)	98.5% (2.8)	100% (0.0)
40,001 and above	7	96.9% (2.2)	92.9% (10.3)	99.9% (0.3)
Total	180	50.9% (42.8)	58.7% (42.8)	72.1% (41.2)

2.3. Antibody identification via Luminex

All serum samples underwent simultaneous antibody identification via a Luminex (LABScreen) HLA antibody screen and HLA antibody identification via multiple single antigen bead assay. These protocols utilized One Lambda PRA beads (LS1PRA and LS2PRA) for screening and One Lambda Single Antigen beads (LS1A04 and LS2A01) for HLA Antibody Identification. Recipient serum samples were incubated with multiple antigen Luminex HLA Class I and II beads (polystyrene microspheres conjugated with fluorochromes, coated with specific HLA molecules), then the sensitized beads were washed to remove unbound antibody. An anti-human phycoerythrin-conjugated IgG was then added to each well (to bind any bound antibody), and then each sample was incubated in the dark on a rotating platform. Each bead mix contained a negative control bead without HLA molecules and a positive control bead with human IgG. Test samples were analyzed via the Luminex instrument, and the signal intensity of each bead was compared to the beads treated with negative control sera.

During Luminex testing, dithiothreitol (DTT) is used to mitigate potential IgM interference via a 1:2 dilution of the sera. DTT was utilized when there was evidence of an interfering substance (such as when the positive control bead fell below a set threshold, or a high MFI negative control result).

HLA antigens of each lymphocyte sample were compared to HLA antibodies identified in plasma samples. When an HLA type matched with an HLA antibody, this was identified as a “Donor Specific Antibody” (DSA). The mean fluorescent intensity (MFI) of each DSA was recorded and a cumulative MFI score for each cell and plasma combination was recorded and quantified.

2.4. Scatterplots and nonlinear regression analysis of data

In order to determine the degree of correlation and relationship between DSA MFI of the Luminex assay and probability of a CAP positive physical XM, we devised scatterplots with Y axes being the percentage of participating laboratories reporting a positive crossmatch and the X axis value being the DSA MFI (either the cumulative MFI of all DSA’s, or the MFI of the largest MFI DSA) that was resulted by our own HLA laboratory. Best-fit lines were fitted and regression with nonlinear allosteric sigmoidal curves via GraphPad Prism software (La Jolla, California).

2.5. Determining HLA group-specific risk

In order to determine whether individual HLA antigen-antibody interactions yielded a greater probability of a positive physical crossmatch, we evaluated the error of prediction for each DSA HLA type. This was performed by calculating the predicted probability of a positive XM, based on the MFI of the top DSA of each XM and the regression