Discordance between Flow-Cytometry Crossmatch and Single Antigen Bead (SAB) Assay: An Uncommon Finding and its Resolution

Sir,

An acute or hyper-acute rejection in any solid organ transplant is due to the presence of preformed anti-HLA antibodies.[1] These antibodies can be identified with cell-based assays; Complement-dependent Lympho-Cytotoxicity Crossmatch (CDCXM) and Flow Cytometry crossmatch (FCXM) and bead-based assays; Panel Reacting Antibodies (PRA) and Single Antigen Bead (SAB).[2] Using cell-based and bead-based assays in an algorithmic manner combines relative merits of each assay to our advantage and allows better interpretation of results. Routinely, in a pre-renal transplant work-up, commonest scenarios are where cell-based crossmatch and SAB are concordant, either negative or positive. We present a case that belongs to a third scenario, an unusual presentation, where CDCXM and FCXM were negative; SAB was positive and virtual crossmatch revealed Donor Specific Antibody (DSA).

A 25-year-old male patient suffering from end-stage renal disease was referred to our laboratory by the nephrologist for pre-transplant workup with his wife as prospective donor. As per the institutional protocol, low resolution HLA typing for class I (A and B) and class II (DR) antigens {polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSOP)} was performed for assessing relationship as a pre-requisite according to The Human Organ Transplant Act, India, 1994 and its amendments.[3] Anti-HLA antibody detection was negative by CDCXM and FCXM. CDCXM was performed using the standard National Institute of Health (NIH) technique and FCXM was performed for T-cell and B-cell on BD FACSVerse™ Flow cytometer (San Jose, CA, USA). Since the patient had history of blood transfusion, PRA was performed using Flow PRA Class I & II Screening Test kit (One Lambda, Inc., CA, USA) and it was found positive. As this scenario presented a discrepancy between cell-based and bead-based methods, repeat testing were done to rule out any technical errors. However, results remained the same. Decision was taken to perform SAB assay (LIFECODES LSA™ Kit, Immucor Transplant Diagnostics Inc., USA) on Luminex platform. SAB assay was positive for class I antibodies and negative for class II antibodies. Antibodies were identified against HLA-A*24:03 (MFI-11531) and HLA-A*24:02 (MFI-5252). Low resolution typing identified donor HLA-A allele as A*24 only. Therefore, high resolution typing for donor HLA-A locus was also done to identify complete antigen. High resolution typing revealed HLA-A*24:03 in donor and confirmed the presence of DSA.

To further understand and resolve this uncommon discrepancy between cell-based crossmatch and SAB, literature was reviewed.[4] Of all the possible mentioned reasons for such discordance, performing tests to negate pro-zone and post-zone phenomenon was undertaken and FCXM was repeated with dilutions of recipient's serum and donor's cells (dilutions 1:2 to 1:8). FCXM was found to be positive for T cells (median channel shift was 59; cut-off ≥26) and negative for B cells (median channel shift was 98; cut-off ≥110) with donor cells in 1:2 dilutions [Table 1 and Figure 1]. This positive result for T-cell FCXM corroborated with SAB results and resolved the discrepancy.

All tests, CDCXM, FCXM, PRA, and SAB, are used to detect the presence of donor-specific anti-HLA antibodies (DSA) in the recipient and in most of the cases, results of all tests are in concordance. However, rarely there can be discordance. Literature review identified following reasons for discordance: Pro-zone/Post-zone effect, stability of antigens, antibody against denatured antigens v/s native antigen and allelic expression on the donor cell surface.

In the present case, post-zone phenomenon was responsible for the discordance between results of cell-based and bead-based assays. Excess of antigen inhibits lattice formation and subsequent agglutination between antigen-antibody may not occur, which can give

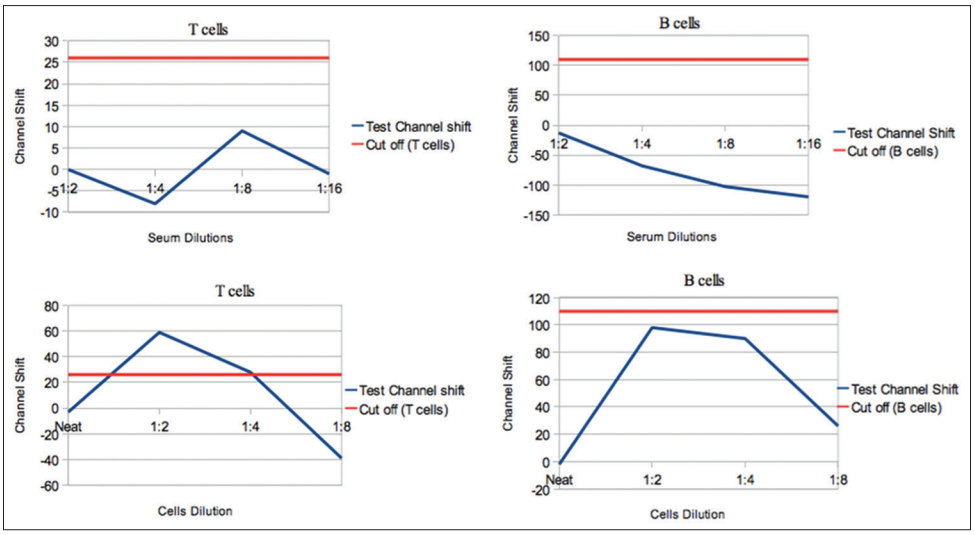

Figure 1: Flow Cytometry Crossmatch results at different serum and cell dilutions

Table 1: Brief Case Illustration

			HLA typing			Results	CDC	FCXM		SAB (donor specific antibody)			
								T-cell	B-cell	Class I		Class II	
Case	Recipient		A	B	DR	Initial	Negative	Negative	Negative	Alleles	MFI	Alleles	MFI
	Age/Gender	Sensitization	11,68	52,52	04,14					HLA-A* 24:03	11531	No DSA	NA
	25/M	Yes (blood transfusion)											
	Donor		A	B	DR	Serum dilutions	Negative	Negative	Negative				
	Age/Gender	Relationship	03,24	55,55	11,14								
	26/F	Wife				Cell dilutions	Negative	Positive	Negative				

CDC=Complement Dependent Cytotoxicity Crossmatch, FCXM=Flow Cytometry based Crossmatch, DSA=Donor Specific Antibodies

false-negative results. Diluting the donor cells or increasing the serum-to-cell ratio can solve the problem.

The case also highlights the importance of performing cell-based and bead-based assay, in an algorithmic manner, in a pre-transplant work-up to rule-out any donor specific antibodies, especially if the recipient has history of any sensitizing event. It can be concluded that in these cases where cell-based assays results are discordant with the SAB assay results, post-zone effect should also be considered to confirm or rule out DSA.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

Simmi Mehra, Aseem Kumar Tiwari, Geet Aggarwal, Dinesh Arora, Abhishek Saini, Chhavi Rajvanshi, Poonam Kumari, Pranav Dorwal[1]

Department of Transfusion Medicine, Medanta-The Medicity, Sector-38, Gurgaon, Haryana, India, [1]Section Head, Flow Cytometry, Waikato Hospital, Hamilton, New Zealand

Address for correspondence:
Dr. Aseem Kumar Tiwari,
Department of Transfusion Medicine, Medanta-The Medicity, Sector-38, Gurugram - 122 001, Haryana, India.
E-mail: aseemtiwari@gmail.com

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