Evaluation of Flow Cytometric Crossmatch for Compatibility of Living Related Bone Marrow Donors

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Abstract

Donor specific antibodies (DSA) are a risk factor in transplantation associated with graft failure in HLA mismatched hematopoietic cell transplants (HCT). However, the solid phase assays for detection of HLA antibodies have limitations, including high background which has the potential to prevent weaker antibodies from being detected, and interfering substances such as medications, resulting in false positive results. In this case, the HCT candidate's anti-HLA antibody profile could not be defined by the single antigen bead assay (One Lambda) due to a high negative control bead (NC) value, reducing sensitivity in the assay. (Fig.1) However, anti-HLA antibodies were a concern as the patient had been receiving frequent transfusions of blood products.

Methods

Compatibility of three potential living related donors, all sons, was assessed by flow cytometric crossmatch (FCXM) to determine the presence of DSA. Crossmatches were run on the CytoFLEX Flow Cytometer by Beckman Coulter.

Qiagen blood kits were used to extract DNA from whole blood on the EZ1 Advanced automated system. NGS-based HLA genotyping was obtained using AlloSeq Tx17 kits from CareDx on the Illumina Miseq instrument for the potential recipient and the three sons (Fig.3). Analysis of the typing results was done utilizing AlloSeq Assign.

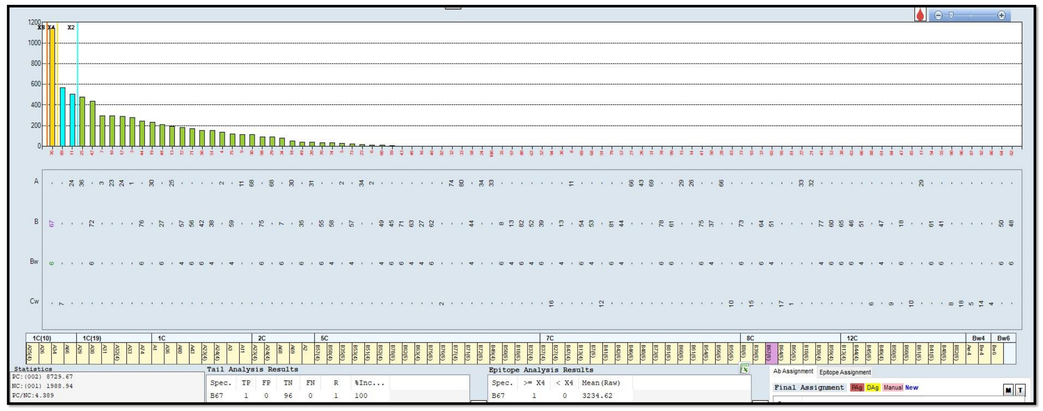


Figure 1. *Class I single antigen bead results in HLA Fusion 4.4 Software. The sample NC value = 1988.94. The laboratory's cutoff for a valid NC is 400.*

Crossmatch

Donor Name:	Donor, One		Crossmatch Result:	Compatible
Relationship:	Son			
	Method	Cell	Result	
	FLOWAB	T	Negative	
	FLOWAB	B	Negative	
Crossmatch Comments:	o No reactivity detected in the flow crossmatch.			
Donor Name:	Donor, Two		Crossmatch Result:	See Comment
Relationship:	Son			
	Method	Cell	Result	
	FLOWAB	T	Positive	
	FLOWAB	B	Wk Positive	
Crossmatch Comments:	o T and B-cell reactivity detected in the flow crossmatch may be attributed to donor specific antibodies (DSA). o Due to high background in the SAB assay, DSA specificities cannot be determined.			
Donor Name:	Donor, Three		Crossmatch Result:	See Comment
Relationship:	Son			
	Method	Cell	Result	
	FLOWAB	T	Wk Positive	
	FLOWAB	B	Equivocal	
Crossmatch Comments:	o T and B-cell reactivity detected in the flow crossmatch may be attributed to donor specific antibodies (DSA). o Due to high background in the SAB assay, DSA specificities cannot be determined.			

Figure 2. *FCXM results identifying Donor, One as a DSA-negative donor.*

HLA Typing Results

	A*	B*	C*	DRB1*	DRB3*	DRB4*	DRB5*	DQB1*	DQA1*	DPB1*	DPA1*
Patient	31:08 11:01	27:05 51:01	02:02 12:03	01:01				05:01	01:01	09:01 10:01	02:01
Relation: Patient											
	31:08	27:05	02:02	01:01				05:01	01:01	09:01	02:01
Donor, One	02:01	51:01	01:02	11:01	02:02			03:01	05:05	104:01	01:03
Relation: Son											
Mismatches:	1		1	1				1			
Donor, Two	01:01 11:01	08:01 51:01	07:01 12:03	03:01 01:01	01:01			02:01 05:01	05:01 01:01	01:01 10:01	02:01
Relation: Son											
Mismatches:	1	1	1	1				1			
Donor, Three	31:08 01:01	27:05 08:01	02:02 07:01	01:01 03:01	01:01			05:01 02:01	01:01 05:01	09:01 01:01	02:01
Relation: Son											
Mismatches:	1	1	1	1				1			

* HLA Typing performed using the following molecular method: NGS.

Figure 3. *The NGS-based HLA genotyping results for recipient and three potential living related donors. Donor, Two and Donor, Three share a haplotype not present in Donor, One.*

Results

Two sons demonstrated positive T and B-cell reactivity, while one son had a negative T and B-cell crossmatch (Fig.2).

NGS-based HLA genotyping was completed on the potential recipient and the three sons. The two sons with positive flow crossmatches shared a haplotype that was not present in the other son. The negative flow crossmatch led to the determination of the third son being a compatible donor.

Conclusions

We concluded the recipient had DSA to one or more antigens in the haplotype shared by two of the three sons, which could not be detected by solid phase HLA antibody testing. FCXM was utilized to help identify a DSA-negative donor. FCXM can be a valuable tool to test for compatibility beyond solid organ transplants when solid phase assays for DSA are inconclusive.

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