CAN MEDIAN FLUORESCENCE INTENSITY OF HLA ANTIBODY DETECTED BY LABSCREEN SINGLE ANTIGEN BEAD ASSAY ABSOLUTELY PREDICT C1Q ACTIVITY?

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AIM

C1q testing has been used in assisting the determination of immunologic risk for HLA antibody, especially when the median fluorescence intensity (MFI) of antibody from LABScreen Single Antigen Bead (LSAB) assay is high. We parallel tested 46 sera by LSAB and C1q assays (One Lambda, Los Angeles, California) to evaluate the correlation between antibody MFI and C1q status. Overall, the results showed: 8%, 50%, and 89% of positive C1q correspond to MFI <10,000, 10,000 ≤ MFI <15,000, and MFI ≥ 15,000, respectively (Table 1). We further explored the potential causes for the low frequency scenarios: high MFI antibodies with negative C1q and low MFI antibodies with positive C1q by the following two case studies.

CASE 1

A 45-year old female heart transplant (Tx) candidate with strong (≥ 15,000 MFI) class I antibodies (PRA = 97%). Unexpectedly, C1q demonstrated negative reactivity (PRA = 0%). Dilutions and additional reflex testing excluded the possibilities of assay saturation (LSAB) and immune-complex blocking (C1q). IgG subclass testing showed positive results for IgG1-4 (Table 2). Most likely, the isoforms of IgG2 and IgG4 diluted the C1q binding capability of IgG antibodies.

CASE 2

A 63-year old male lung Tx recipient with pre-Tx negative T and B cell flow cytometry crossmatch and a possible borderline (cut off ≥1000 MFI) class I donor specific antibody (DSA). De novo DSA to DR8 (MFI = 2837) has been detected; however, this low MFI DR8 DSA was C1q positive. Heat and additional reflex testing ruled out the interference of IgM and immune complex of the C1q assay. Interestingly, IgG subclass testing indicated that IgG3, the IgG isoform with the strongest complement binding ability among IgG1-4, was the only IgG isoform of DR8 DSA (Table 2). Most likely, the dominant isoform of IgG3 enhanced the C1q binding capability of DR8 DSA.

Table 1. The Correlation between the Median Fluorescence Intensity (MFI) from LABScreen Single Antigen Bead (LSAB) Assay and C1q Status

	LSAB		
	MFI < 10,000 (n = 12)	10,000 ≤ MFI <15,000 (n = 16)	MFI ≥ 15,000 (n = 18)
Positive C1q % (n)	8% (1)	50% (8)	89% (16)

Table 2. Case Studies on the Correlation of HLA Antibody Testing Results by LABScreen Single Antigen Bead (LSAB), C1q, and IgG Subclass Assays

	Case Status	LSAB	**C1q	***IgG Subclass			
Case 1	Heart transplant (Tx) candidate who has strong Class I antibodies from pre-Tx sample testing	*PRA (%) MFI ≥ 15,000	PRA (%) MFI ≥ 1,000	PRA (%) MFI ≥ 1,000			
				IgG1	IgG2	IgG3	IgG4
		97	0	98	98	95	95
Case 2	Case Status	LSAB	C1q	IgG Subclass			
	Lung Txed recipient who has Class II De Novo DSAs from post-Tx sample testing	De Novo DSA MFI ≥ 10,000	De Novo DSA MFI ≥ 1,000	De Novo DSA MFI > 1,000			
				IgG1	IgG2	IgG3	IgG4
		None	DR8	None	None	DR8	None

*PRA: panel-reactive antibody
**C1q and ***IgG Subclass: cut off of these two assays is MFI ≥ 1,000

CONCLUSIONS

High MFI values of HLA antibody detected by LSAB correlates to high possibility of positive C1q and most often can be used to predict C1q reactivity. Due to the occurrence of exceptional cases that are possibly related to the varying IgG subclass ratios, the C1q assay is still highly recommended to evaluate the immunologic risk for HLA antibody.

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