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A study of living donor renal transplantation with inconsistent flow cytocross-match (FCXM) and preformed donor-specific antibodies (DSA)

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COI Disclosure Information

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I have no relevant financial relationships to disclose.

Introduction

The approach to preoperative crossmatch-positive patients for living donor renal transplantation is difficult because the presence of preformed donor-specific antibodies (DSA) and crossmatch test results can be inconsistent; consequently authenticity of the test results and immunological background must be carefully considered in each case. In this study, we investigated the presence of DSA, desensitization therapy, rejection, postoperative DSA status, and transplant renal function in living donor renal transplant recipients with positive preoperative flow cytocross-match (FCXM) results.

Method

Of the 195 living donor renal transplant cases performed in our department since 2016

32 cases (16.4%) **of positive FCXM**

	FCXM-B positive	FCXM-B negative
FCXM-T positive	**6** (3.1%)	**9** (4.6%)
FCXM-T negative	**17** (8.7%)	

All of these cases are complement dependent cytotoxicity (CDC) negative.

Result (1)

FCXM-T only positive cases

	Donor	PF-DSA HLAclass Ⅰ	PF-DSA HLAclass Ⅱ	ABO incompatibility	preoperative			postoperative days	Cr(mg/dL) at 1year	latest Cr (mg/dL)	De-novo DSA	First year transplant kidney biopsy
					Rituximab	DFPP	IVIG					
1	wife	**NEG**	**NEG**	compatible	–	done	done	367	**0.77**	**0.77**	–	no rejection
2	wife	**NEG**	**NEG**	compatible	–	done	done	374	**1.74**	**1.74**	–	no rejection
3	husband	**NEG**	**NEG**	A→**O**	done	–	–	990	**1.71**	**1.75**	–	IF/TA
4	husband	**NEG**	**NEG**	B→**O**	done	done	–	1417	**1.65**	**1.48**	–	arteriosclerosis
5	mother	**NEG**	**NEG**	A→**O**	done	–	–	1557	**2.37**	**2.79**	–	IF/TA, arteriosclerosis
6	mother	**NEG**	**NEG**	A→**O**	done	done	–	1683	**2.05**	**2.07**	–	no rejection
7	husband	**NEG**	**NEG**	A→**O**	done	done	–	2453	**0.68**	**1.25**	–	IF/TA, no rejection
8	wife	**NEG**	**NEG**	B→**O**	done	done	–	2740	**0.87**	**1.2**	–	no rejection
9	sister	**NEG**	**NEG**	compatible	–	–	–	2747	**0.64**	**0.64**	–	Not implemented

There were 9 cases that tested positive only for T, a group that could theoretically be considered a technical error. It is interesting to note that most of the patients were type O recipients. This corresponded to about 8% of the type O recipients performed at our institution.

FCXM-T,-B both positive cases

	donor	FCXM T cell	ratio	FCXM B cell	ratio	PF-DSA HLA class Ⅰ	PF-DSA HLA class Ⅱ	ABO incompatibility	preoperative			postoperative days	Cr(mg/dL) at 1-year	lately Cr (mg/dL)	DSA	First year transplant kidney biopsy
									Rituximab	DFPP	IVIG					
1	mother	+	3.04	+	3.37	NEG	NEG	B→**O**	done	done	done	234	-	1.34	NEG	no rejection
2	wife	+	2.04	+	1.68	NEG	NEG	B→**O**	done	done	-	Nephrectomy due to non-immunological reasons				
3	daughter	+	6.1	+	2.5	B62 (2108)	NEG	compatible	done	done	done	696	0.42	0.42	disappearance	no rejection
4	son	+	2.96	+	2.91	A24 (1819) B54 (3661)	NEG	compatible	done	done	done	843	0.5	0.51	A24(518) B54(1439)	antibody-mediated rejection
5	husband	+	1.56	+	2.03	NEG	DR4 (1993)	compatible	done	done	done	934	0.95	0.98	disappearance	antibody-mediated rejection
6	husband	+	1.5	+	2.6	NEG	DR4 (1580)	compatible	done	done	done	1235	0.92	1.09	DR4(2004)	antibody-mediated rejection

Theoretically, this group should have at least a DSA for class 1, so the only cases that are consistent are 3 and 4. However, in cases 1, 2, 5, and 6, DSA to class 1 was not detected, and the results of FCXM and DSA were clearly inconsistent. Since cases 1 and 2 have a false-positive pattern specific to type O recipients, and since there is no DSA for class 2, even though they are FCXM-B positive, I have come to believe that they can be ignored here. For Cases 5 and 6, the positive FCXM-T results can be considered a discrepancy. In the case with a history of sensitization from husband to wife, a class 2 DSA was detected, so we did not hesitate to administer desensitization therapy.

Result (2)

FCXM-B only positive cases

	donor	PF-DSA(MFI)	ABO incompatibility	preoperative			postoperative days	1-year Cr	latest Cr	De-novo DSA	Renal biopsy
				RTx	DFPP	IVIG					
1	father	NEG	compatible	-	-	-	206	—	1.68	NEG	
2	husband	NEG	A→O	done	done	-	626	0.67	0.67	NEG	
3	**wife**	**DQ6 (8000)**	compatible	done	done	**-**		**PNF**		N/A	**Hyperacute antibody-mediated rejection**
4	mother	NEG	compatible	-	-	-	1242	1.97	2.29	NEG	
5	wife	NEG	compatible	-	-	-	1522	1.91	2.74	NEG	Suspected chronic T-cell rejection
6	husband	NEG	compatible	-	-	-	1585	1.06	0.97	NEG	
7	wife	NEG	compatible	-	-	-	1599	1.51	1.41	NEG	
8	sisiter	NEG	compatible	-	-	-	1662	1.15	1.03	NEG	
9	wife	NEG	AB→A	done	-	-	1697	1.54	1.8	NEG	
10	mother	NEG	compatible	-	-	-	1718	1.13	1.06	NEG	
11	mother	NEG	compatible	-	-	-	1739	1.66	1.36	NEG	
12	mother	NEG	A→O	done	-	-	*1753*	*3.12*	*HD*	NEG	acute antibody-mediated rejection
13	brother	NEG	compatible	-	-	-	1774	0.92	0.83	NEG	
14	husband	NEG	A→B	done	-	-	2089	1.06	1.11	NEG	
15	mother	NEG	A→O	done	done	-	2201	1.06	1.08	NEG	
16	mother	NEG	compatible	-	-	-	2684	1.38	1.45	NEG	
17	**husband**	**DR9 (2419)**	compatible	done	done	**-**	**2761**	**1.01**	**1.73**	disappearance	**arteriosclerosis, medullary-radial injury**

PF-DSA: preformed Donor specific antigen RTx: rituximab NEG: negative DFPP: double filtration plasmapheresis IVIG: Intravenous Immunoglobulin PNF: primary non function IF/TA: interstitial fibrosis and tubular atrophy

The two cases with PF-DSA were blood group-matched transplants, so desensitization with rituximab and DFPP was administered. In the other cases, transplants were performed without desensitization except for blood group incompatibility transplants.

Why do the flow cytocross match results not match the HLA antibody results?

The interpretation of FCXM positives can be categorized as shown in the table, but the important thing is not to simply judgethem as T or B negative or positive, but to judge them in combination with the results of both. In this article, we have classified the 32 positive cases accordingly.

	FCXM-B positive	FCXM-B negative
FCXM-T positive	DSA HLA class I only or HLA class I and II	Technical Error
FCXM-T negative	DSA HLAclass II	No DSA

- In FCXM-B positivity, the DSA positivity rate was only about 11%, and conversely, 90% of the patients did not have DSA. This is due to a nonspecific reaction caused by the Fc receptor. In this study, we believe that no special desensitization is necessary for cases in which DSA was not observed.
- However, under certain conditions, the risk of acute antibody-associated rejection is increased due to the expression of class 2 antigens in the vascular endothelium, so adequate desensitization is necessary if DSA is detected.

- Approximately 10% of type O recipients tested positive only for flow cytocross match T. This is considered a false positive reaction caused by the reaction of anti-A and anti-B antibodies to blood group antigens on T lymphocytes.
- Therefore, if DSA is negative, desensitization equivalent to that of a normal blood group incompatible transplant is likely to be sufficient.

- Considering that FCXM-B is prone to false positives in the first place, it is possible that a type O recipient who is positive for both FCXM-T and B is simply a false positive for each.
- In one of our cases, the recipient was desensitized to the usual blood group incompatibility transplantation and has continued to do well postoperatively.

False positives may be caused by reaction of ABO blood group antigens on T lymphocytes with anti-A and anti-B antibodies of type O recipients.

Oriol R et al.: Am J Hum Genet, 1981; 34: 551–560.
Rachkewich RA et al.: J Immunogenet, 1978; 5: 25–29.
Bernoco M et al.: Vox Sang, 1985; 49: 58–66.

Nonspecific reactions due to Fc receptors increase the likelihood of false positives for FCXM-B

Vaidya S, Transplantation, 71, 422-428, 2001

In humans, cytokine stimulation induces expression of MHC class II molecules in non-immune cells such as vascular endothelial cells.

Hiwa R, Jpn. J. Clin. Immunol.,39, 78-83,2016

Conclusion

- ✓ A positive flow cytocross match in renal transplant patients is difficult to interpret the results, so careful consideration must be given to planning desensitization therapy.
- ✓ Based on the FCXM and DSA results, we were able to divide the cases into groups with certain tendencies, and we were able to find discrepancies in these results, i.e., the possibility of false positives.
- ✓ The results were considered to be helpful in planning and formulating desensitization therapy without excesses or deficiencies.