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HLA-DP antibody leading to positive crossmatch: Rare occurrence

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Abstract

Background: In order to improve the success rate for transplantation the combination of assays have been used in which the flow based methods carry high degree of sensitivity but significance of positive B Cell Flow crossmatch (FCXM) remains controversial.

Case Report: Our case presents a 31 years old male with no history of previous transplant, but having sensitisation history of 6 units of whole blood and 5 units of packed red blood cells transfused two month before presentation. The prospective donor was the brother of the patient. He was recommended Complement Dependent Cytotoxicity cross Match (CDCXM), FCXM and Flow Panel Reactive Antibody (PRA) and Human Leukocyte Antigen (HLA) Typing (A, B, DRB1) locus, as part of pre-transplant work-up. CDCXM and Flow PRA were found to be negative, while FCXM was B cell positive. The repeat testing for FCXM had similar results. Finally, it was decided to perform Luminex based Single Antigen Beads (SAB) assay. A total of 13 anti-HLA antibodies were identified in the patient serum, while only one of these being a Donor Specific Antibody (DSA, DPA1*02:01).

Conclusion: Positive B cell FCXM results should not be overlooked and whenever possible should be followed with DSA by SAB assay to increase the sensitivity of the screening panel of the patients before transplantation to enhance the graft survival and lower the rejection rates

Keywords

HLA DP; CDCXM; FCXM; PRA; DSA

Introduction

It has been long recognized that the pre-formed donor specific antibodies directed against HLA (Human Leukocyte Antigen) is the main cause for antibody mediated rejection and poor graft outcome.

Crossmatch was developed by Terasaki in 1969 to identify patient with a high likelihood of developing hyper acute rejection [1,2]. Cross matching has been a mandatory component of renal transplant work up for over 40 years now and has led to a substantial reduction in hyper-acute rejections. Crossmatch techniques have widely evolved over the years, but the basic principle behind all these technologies is to determine if donor specific antibodies are present in the recipient serum.

Currently, there are various cross match techniques available ranging from the basic complement dependent cytotoxicity crossmatch (CDCXM), flow crossmatch (FCXM) and virtual crossmatch via Luminex. Regardless of the technology, as per the consensus guidelines of 2013 transplantation risk stratification categories should be developed based on antibody identification and XM results and all the results should be interpreted in-Toto [3].

Flow cytometry as a tool in solid organ transplantation has evolved significantly for the past many years as it also facilitate the distinction between T cell and B cell reactivity [4]. This helps in determining the increased sensitivity of flow and early prediction of graft failure and increased episodes of rejection.

Positive T cell FCXM is proven to be associated with higher graft rejection rates and lowers graft survival [5,6] while there is still a clinical dilemma about the positive B cell FCXM [7,8]. Thus, detailed follow-up of patients with positive B cell FCXM along with one of the SPI (solid phase immunoassay) to pick up rare antibodies may help to improve graft survival and reduce the rate of graft rejection

Case Report

A pre-transplant works up was performed for a 31 years old male, who had history of blood transfusions two months prior to the sampling. The patient had no history of previous transplant and the prospective kidney donor was the sibling (brother) of the patient. The patient had undergone dialysis 3 days prior to the sampling and the test ordered by the transplant physician for this work up included CDCXM, FCXM and Flow PRA.

Results

CDCXM (including AHG enhancement) showed <10% cell death in neat and subsequent dilutions of patient serum. We scored as per the American Society of Histocompatibility and Immunogenetics (ASHI) guidelines, with the negative control and media control with <10% of cell death scored as 1, the positive control with >80% of cell death was scored as 8.

In the Flow PRA screening test, the percent of PRA for Class I was 2% and for Class II was 3% which as per the reporting criteria and the cut-off set for our laboratory reported as negative (Figure A).

In the FCXM, T cell showed no shift in IgG channel and fluorescence index (FI) was 0%, which was considered as negative. B cell IgG channel showed significant shift with FI of 14% which was reported as positive FCXM for B Cell (Figure B).

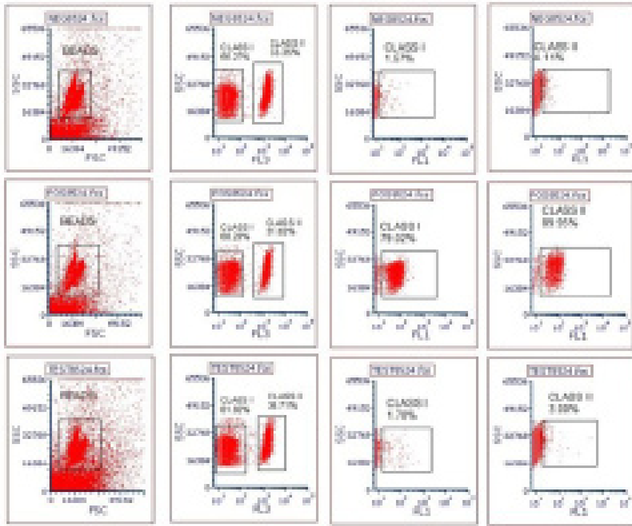

Figure 1: Flow PRA

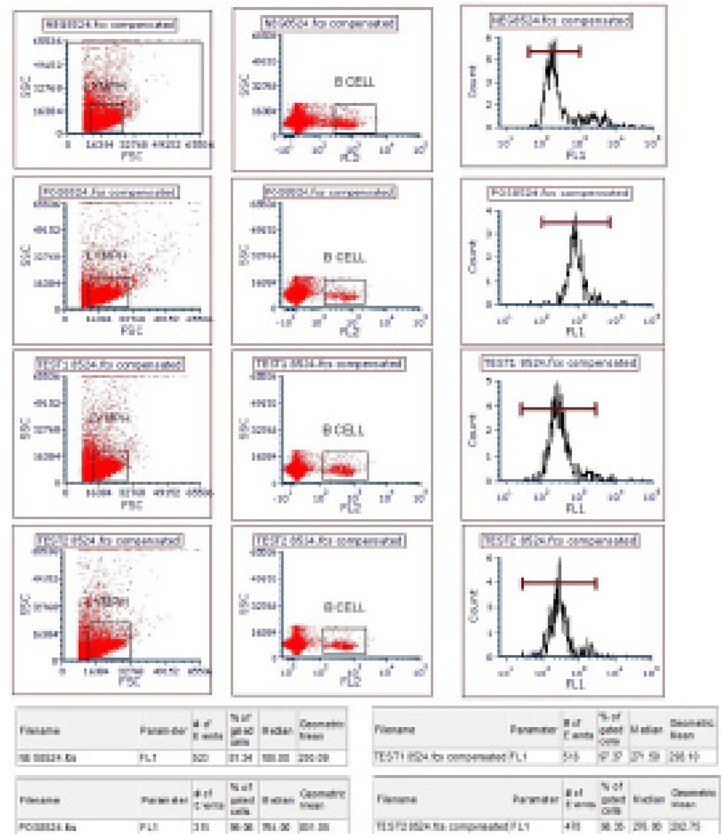

Figure 2: FCXM T and B cell

Low resolution Sequence specific oligonucleotides probes (SSOP) technology on Luminex was also performed for HLA A, B, DR locus of the potential donor (Table 1).

In view of B cell FCXM positivity and negative flow PRA, Single Bead Assay (SAB) on Luminex platform was performed using Immucor kits for Class I and II was performed. We found 7 anti HLA antibodies against Class I and 6 Class II anti-HLA antibodies (Table 2).

High resolution HLA typing of DPA1 and DPB1 locus was performed for the case as SAB assay revealed antibodies against them (Table 3).

There was one DSA identified against DPA1*02:01 which was present after comparing all the results of SAB and high-resolution typing. This explains B cell FCXM positivity in this case.

Tables

	A locus	B Locus	DR Locus
Patient	A*24, 24	B*40, 40	DRB1*03,15
Donor	A*32, 11	B*52, 52	DRB1*15, 10

Table 1: HLA low resolution typing of Donor

Class I		Class II
A Locus	B Locus	DPA DPB
A*31:01	B*13:02	DPA1*04:01-DPB1*04:01
	B*15:03	DPA1*02:01-DPB1*04:02
	B*15:13	DPA1*02:02-DPB1*04:01
	B*45:01	DPA1*02:02-DPB1*28:01
	B*48:01	DPA1*02:01-DPB1*17:01
	B*27:05	DPA1*02:01-DPB1*05:01

Table 2: SAB assay for Class I and Class II

DPA1 Locus	DPB1 Locus
DPA1*02:01	DPB1*04:02:01G
DPA1*01:03	DPB1*26:01:02

Table 3: HLA typing of DPA1 and DPB1

Discussion

Considering the high sensitivity of FCXM as compared to CDC, it has become a crucial investigation in renal pre-transplant individual's workup. The principle behind the CDC and FCXM is similar with only difference been that in FCXM the antigen - antibody reaction is detected via fluorescein labelled antibodies specific against Fc part of human IgG. FCXM also has the benefit of having less subjective variations. A Negative result for both T and B cell indicates that the patient serum is unlikely to have donor specific antibodies. There has been contrasting reports on the significance of B cell positivity, however, an isolated and strong B cell positivity shouldn't be overlooked as is it can be associated with class II antibodies. [9,10] T cell positivity, on the other hand is considered to be extremely important as it has shown to have an impaired long term graft survival even with a negative CDC [11].

Flow PRA has high HLA specificity, because the measure of PRA is dependent on the composition of the HLA antigen panel, it is possible that the panel may not reflect the antigen frequencies in the donor population and hence, may not be a good reflection of the chances of a patient finding a compatible donor [1,9,18].

The present case had negative T cell FCXM while B cell FCXM was positive and after thorough workup the DSA for Class II antigen i.e. DPA1 was detected on SAB assay by Luminex. Zhang et al have stated that the DP DSA when combined with other HLA DSA demonstrate adverse clinical outcome, however its significance on its own is not well documented [12]. This antibody can be picked by FCXM positivity for B cell. As the antigen density on SAB beads are higher than Flow PRA beads [13] the two different platforms should be used to work up the pre-transplant patient to detect these clinically significant rare anti HLA antibodies . Flow PRA can be used to gauge the degree of sensitisation in a patient, while

Tambur et al in 2014 reported that 320 out of 2948 FCXM performed were positive because of HLA DP donor specific antibodies and 58/207 (12%) HLA DR serologically matched donor recipient pairs had

a positive B cell FCXM, thus proving the importance of positive B cell FCXM positivity and complete donor HLA typing at time of organ donor in pre transplant workup [14].

FCXM is sensitive and versatile test to detect Donor specific antibody (DSA) as a primary test [15]. Our study supports the study done by Lionel Couzi et al [16] that showed increased sensitivity and clarity on sensitization status in renal trasnplant patients by addition of SAB assay with FCXM test in the pretransplant as well as post tranplant workup .

One of the study done by Gloor et al on 189 patients which included 119 positive XM and 70 negative XM cases investigated using FCXM as well as AHG-CDC and DSA techniques also concludes the importance of combination of the techniques in prediction of detection of risk of rejection of graft. All these techniquies should be used in pretransplant screening as well as in post transplant monitoring of the patients.He concluded that combining all the techniques helps in detecting and reducing risk for both early and late allograft loss [17].

Conclusions

From the above case we would like to conclude that, the positive B cell FCXM results should not be overlooked and whenever possible should be followed with DSA by SAB assay.Thus,increasing the sensitivity of the screening panel of the patients before transplantation to improve graft survival

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