Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/ijt

# The importance of donor-specific anti-HLA antibodies (DSA) identification in renal transplant patients with C4d-negative biopsies

S. Mehrotra [a], R.K. Sharma [a,*], N. Prasad [a], A. Gupta [a], D.S. Bhadauria [a], A. Kaul [a], M. Jain [b], P. Rishishwar [c]

[a] Department of Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow, UP, India

[b] Department of Pathology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow, UP, India

[c] Department of Pharmacy, SV University, Gajraula, India

ARTICLE INFO

*Article history:*
Received 14 September 2015
Received in revised form
24 February 2016
Accepted 1 March 2016
Available online 11 March 2016

*Keywords:*
C4d staining
Panel reactive antibody
Complement dependent cytotoxicity

ABSTRACT

Two index cases of living-related donor renal allografts patients developed C4d-negative rejection. Both cases had negative cytotoxic crossmatch and negative flow crossmatch before transplantation. The serum creatinine levels were tabulated. Both cases experienced augmented anti-T cell therapy (intravenous methyl prednisolone) at the time of rejection, which failed to improve renal function. Meantime, our HLA lab identified circulating anti-class I and/or II HLA antibodies towards donor mismatched antigens by Luminex multiplex bead array. Additional therapy included high-dose IVIg and plasma exchange. The renal function improved significantly. Furthermore, the donor-specific antibody strength decreased after combined plasmapheresis and IVIG therapy. These cases highlight the importance of donor-specific antibody detection by sensitive solid phase assays in the context of C4d-negative ABMR.



## 1. Introduction

The presence of C4d sub-endothelial deposition has been considered a major diagnostic element of antibody-mediated rejection (ABMR) after renal transplantation. More than 60% of late antibody mediated rejection (ABMR) is C4d negative.[1] Particularly important for the Indian setting is the practice of not routinely measuring the DSA by single antigen bead (SAB) assay at the time of graft biopsy done for evaluating graft dysfunction, as is done in western countries.

## 2. Case studies

Two index cases of living-related donor renal allografts patients developed C4d-negative rejection. Both cases had negative cytotoxic crossmatch and negative flow crossmatch before transplantation. The serum creatinine levels are shown in

* *Corresponding author at*: Department of Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow 226014, India. Tel.: +91 9415026762.
E-mail addresses: rksharma@sgpgi.ac.in, rksnepho206@gmail.com (R.K. Sharma).

http://dx.doi.org/10.1016/j.ijt.2016.03.001

**Table 1 – Cases: profile and response to ABMR specific therapy.**

| Case | Creatinine at baseline (mg/dl) | Histology | C4d | Creatinine at rejection mg/dl | Tacrolimus levels at rejection ng/ml | Anti-HLA DSA class I + class II | | Creatinine after PP + IVIG mg/dl |
|---|---|---|---|---|---|---|---|---|
| | | | | | | At time of rejection | Post PP+ IV IG | |
| #1 | 0.92 | Acute T-cell mediated rejection peritubular capillaritis | Negative | 2.03 | 3.2 (normal range 4.0–6.0) | Anti-A 26 (MFI 2673), anti-DQ06 (MFI 1522), anti-DR11 (MFI 1941), anti-DR 13 (MFI 1662), Solid phase DSA: (class I MFI 1187, class II MFI 2438) | Solid phase DSA negative (class I MFI 141, class II MFI 645) | 1.7 |
| #2 | 1.4 | Focal interstitial mononuclear infiltrates with peri-tubular capillary dilatation | Negative | 2.7 | 1.4 (normal range 4.0–6.0) | Anti-B 56 (MFI 1598), anti-DQ03 (MFI 15843) Solid phase DSA: (class I MFI 1403, class II MFI 12740) | Solid phase DSA negative (class I MFI 243, class II MFI 895) | 1.8 |

Table 1. Both cases experienced augmented anti-T cell therapy (Intravenous methyl prednisolone) at the time of rejection, which failed to improve renal function. Meantime, our HLA lab identified circulating anti-class I and/or II HLA antibodies toward donor mismatched antigens by Luminex multiplex bead array. Additional therapy included high-dose IVIg and plasma exchange. The renal function improved significantly. Furthermore, the donor-specific antibody strength decreased after combined plasmapheresis and IVIG therapy. These cases highlight the importance of donor-specific antibody detection by sensitive solid phase assays in the context of C4d-negative ABMR.

## 3. Discussion

Sensitive solid-phase platform allows for better diagnosis of ABMR after renal transplantation by identifying donor-specific anti-HLA antibodies. This is valuable for the clinical transplant team, especially in cases of inconclusive histopathology and C4d-negative histology. The routine monitoring of DSA in the post-transplant setting is a predictor of progression of antibody mediated rejection independent of C4d status (Case 2). These two cases provide an important message for the Indian population; where DSA (SAB) is not routinely measured at the time of transplant biopsy and where C4d staining alone has until recently been the gold standard for the diagnosis of ABMR.

At our center (Sanjay Gandhi Post Graduate Institute of Medical Sciences), living related renal transplant recipients with negative pre transplant CDC crossmatch were evaluated by solid phase cross match (SPC) after transplantation. 21.5% recipients had DSA by SPC and those who were positive for HLA class II antibodies had significant association with ABMR. Solid phase crossmatch (SPC) testing performed with donor lysate on Luminex platform could sever as an estimate for diagnosis of rejection episodes. SAB is gold standard for the diagnosis of ABMR (Figs. 1 and 2).

DSA with single or multiple antigen beads is costly (particularly for individual patients). Solid phase crossmatch (SPC) testing is less expensive than SAB assay. Donor-specific antibody testing by SPC costs Rs. 2800 per test and by SAB assay costs 25,000 per test.

Solid phase crossmatch assay by Luminex can also be used for post-transplant routine DSA monitoring (economical for Indian population, although this test has limitations for the detection of HLA antibodies).

The presence of pre-transplant antibodies directed against donor antigens (HLA) called donor-specific antibodies (DSA) is strongly associated with hyper acute and acute rejection.[2–4] Production of de novo DSA after transplant is indicative of the antibody-mediated rejection (AMR).[5] The HLA-DSA monitoring is an important tool for rejection risk assessment in kidney transplant recipients.[6,7]

### 3.1. *Complement dependant cytotoxicity (CDC) cross match*

This test was first standardized by Terasaki in 1960s.[8] It is used to detect the donor-specific antibodies directed against the recipient. Patel and Terasaki developed the CDC cross match for pretransplant donor recipient evalua-