Complement Fixing HLA-C Antibodies in a Pre-Transplant Lung Patient Sensitized by Pregnancy: To Cross or Not to Cross?

Min-Jeong Lee[1], Ian-Michael Salvador[1] Robert Vorhaben[1], Chantale Lacelle[2]

Clinical Laboratory Services[1] and Department of Pathology[2],
University of Texas Southwestern Medical Center, Dallas, Texas

INTRODUCTION

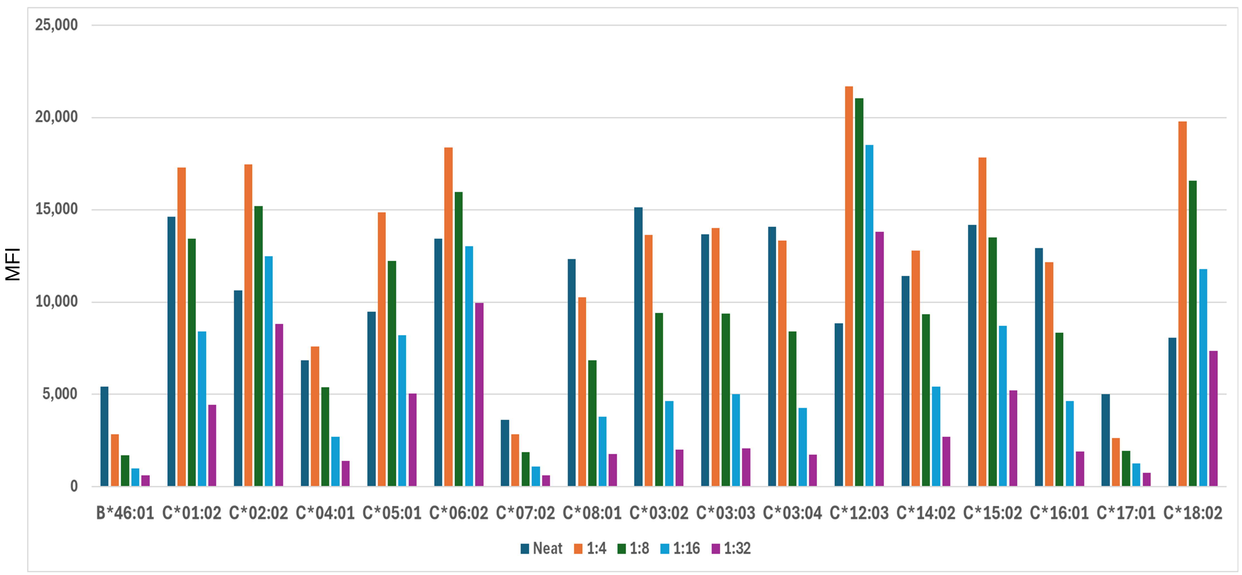
Select patients awaiting lung transplantation present with pan-C reactivity when tested using the One Lambda LABScreen single antigen assay. In most of those patients, the reactivity does not yield positive crossmatches since it is caused by the recognition of an epitope on the surface of beads that is absent from HLA-C antigens expressed on cells. One Lambda has a reagent, PreSorb, which can be used to mitigate this type of reactivity. Recently we identified a patient reacting to all HLA-C beads on the LABScreen single antigen panel despite having relatively few other HLA class I antibodies. In fact, A3 and B46 were the only non-HLA-C beads with signal strength above 5,000 MFI. Given the observed antibody pattern, we suspected this patient may have physiologically irrelevant antibodies which react to beads only. Further investigations were performed to assess the validity and possible impact of the HLA-C antibodies detected by single antigen assay.

METHODS

- **SINGLE ANTIGEN ASSAY:** The patient's sample was tested with the One Lambda LABScreen single antigen assay and data was analyzed using an in house developed software.
- **C1Q ASSAY:** C1q assay was performed using One Lambda C1q test kit and analyzed using the Fusion software.
- **FLOW CYTOMETRY CROSSMATCH:** Flow cytometry was performed using non-pronase T cells.
- **HLA TYPING:** HLA typing for donor and recipients were performed using One Lambda LABType™ XR or in house developed sequenced based typing.

RESULTS

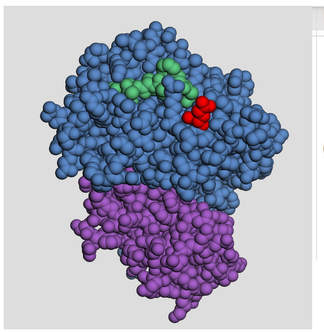
FIGURE 1: Single Antigen Results for HLA-B*46 and all HLA-C Alleles



Result: HLA-B*46 as well as all HLA-C alleles on the One Lambda LABScreen single antigen panels yields positive results with the patient's sample. Serial dilution of the sample was performed up to 1:32. The patient's HLA-C typing is HLA-C*07:01, 07:18. Neither alleles are present on the on the One Lambda LABScreen single antigen panel (regular panel). The majority of HLA-C antibodies are C1q positive.

FIGURE 2: Eplet Analysis Identifies 65QKR in HLA-B*46 and all HLA-C Alleles Present on the Single Antigen Panel

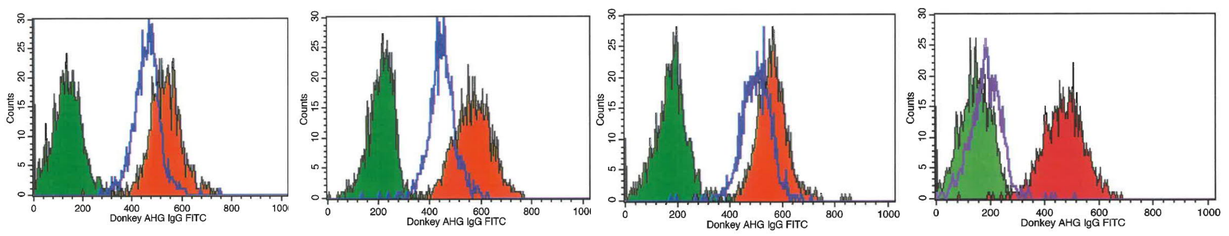
AA Pos.	10	20	30	40	50	60	70	80	90
C*07:02:01:01	CSHSMRYFDT	AVSRPGRGEP	RFISVGYVDD	TQFVRFDSDA	ASPRGEPRAP	WVEQEGPEYW	DRETQKYKRQ	AQADRVSLRN	LRGYYNQSED
C*07:01:01:01	----------	----------	----------	----------	----------	----------	-----N----	----------	----------
C*07:18:01:01	----------	----------	----------	----------	----------	----------	-----N----	----------	----------



Name	Description	Exposition*	3D View	Confirmation	Luminex® Alleles
65QKR	65Q66K69R	Intermediate	View	Yes	B*46:01, C*01:02, C*01:03, C*02:02, C*02:10, C*03:02, C*03:03, C*03:04, C*04:01, C*04:03, C*05:01, C*06:02, C*07:02, C*07:04, C*08:01, C*08:02, C*08:03, C*08:04, C*12:02, C*12:03, C*14:02, C*14:03, C*16:01, C*16:02, C*17:01, C*17:03, C*18:01, C*18:02

Result: 65QKR epitope is absent from the patient's HLA-C molecules. The patient is HLA-C*07:01, 07:18 which has a 65Q66N69R instead of 65Q66K69R sequence. Sequence alignment was generated using IMGT. 3D molecular structures shows the QKR eplet in red. Blue=alpha chain, purple= beta-2-microglobulin and green=peptide. 3D structure was generated using pHLA3D (Oliveira et al. Human Immunol. Vol 80 issue 10, Oct 2019 p 834-841). Epitope description is from the Brazilian HLA epitope registry (HLA Eplet Registry (epregistry.com.br) .

FIGURE 3: Surrogate Donor T cell Crossmatches Results



HLA-C*04:01, 08:01 | HLA-C*03:03, 07:01 | HLA-C*05:01, 18:01 | HLA-C*04:01N, 07:01

GREEN = NEGATIVE CONTROL RED= POSITIVE CONTROL BLUE = PATIENT'S SAMPLE

Result: Four T cell flow cytometry crossmatches were performed using surrogate donor cells. Crossmatches were positive except with donor cells typed as HLA-C*04:01N, 07:01. The negative crossmatch cells do not have the 65QKR eplet.

CONCLUSION

Herein, we present the case of a patient primarily sensitized toward HLA-C. While complement fixing HLA-A and -B, antibodies are generally avoided in lung transplantation, there is little guidance for HLA-C. Due to lower expression of HLA-C on tissues, transplantation across HLA-C antibodies is common. However, no studies have addressed whether transplant across HLA-C complement fixing antibodies is associated with poorer graft outcome in lung transplantation and whether there is a benefit in desensitizing those patients. Obtaining this information will be of great value since it is unlikely these patients will be transplanted with a negative crossmatch without desensitization given the lack of HLA matching in non-renal transplantation.