Acute Heart Transplant Rejection in the Presence of Weak DSA with Negative Flow Cytometry Crossmatch

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BACKGROUND

This case study involves a 10-year-old male who received a heart transplant. Pre-transplant antibody testing on 9/25/23 showed weak donor-specific antibodies (DSA) to HLA-A3 and -A32. At the time of transplant on 10/23/23, low levels of DSA to HLA-A3 and -A32 were detected, and flow cytometry crossmatch results were negative for both T and B cells. At day 7 post-transplant, strong DSAs to HLA-A3 and -A32, along with de novo DSAs to HLA-B7, -B35, -DR4, -DQ8 and -DPB1*06:01, were detected. By day 14 post-transplant, the majority of DSA levels dramatically increased. Although initial plasmapheresis reduced HLA Class I and II DSA MFI values, they rebounded over time. Unfortunately, the patient experienced worsening antibody-mediated rejection (AMR) and tissue damage, leading to graft failure.

AIM

This case study aims to demonstrate that acute rejection of a heart transplant can occur despite the presence of low levels of DSAs and a negative (or weakly shifted) flow cytometry crossmatch at the time of transplantation.

METHODS

- **High-resolution SAB (single antigen bead) test panels for HLA antibody identification**
  Serum was treated with EDTA and Adsorb Out beads. HLA antibody testing was performed using Luminex SAB assays (LabScreen SAB, One Lambda Thermo Fisher) on the Luminex FLEXMAP 3D
- **Low-resolution HLA typing utilizing LABType rSSO DNA typing assays**
  HLA typing of patient was performed utilizing LABType reverse SSO by bead-based multiplexing on Luminex LABScan 3D coupled with HLA Fusion Software for result analysis(LABType rSSO, One Lambda Thermo Fisher)
- **Flow cytometric crossmatching (FCXM) to assess donor cell compatibility with antibodies present in a serum sample**
  The lymphocyte population was obtained from EasySep Direct Human Total Lymphocyte Isolation Kit (STEMCELL Technologies). Donor and recipient cells were treated with Pronase and DNase. Three-color flow cytometry crossmatches were performed on the FACSCanto II Flow Cytometer. Molecules of equivalent soluble fluorochrome (MESF) values were used for FCXM results interpretation.
- **HLA Eplet Registry software was used to perform eplet analysis to investigate shared eplets between pre-existing DSA and de novo DSAs against antigens**
- **C1q testing was performed by another lab**

RESULTS

Negative allogeneic FCXM with weak HLA-A3 and -A32 DSA at the time of transplant

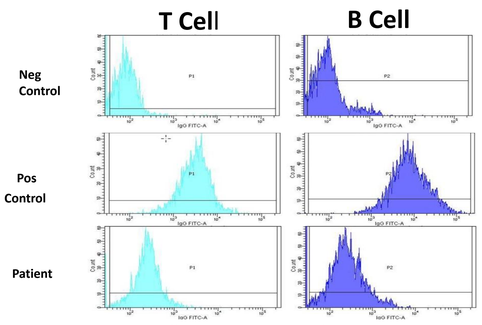

Figure 1. Allogeneic FCXM results

Method	T cell		B cell	
	Delta MESF	Interpretation	Delta MESF	Interpretation
Allogeneic FCXM	3220	Neg	2744	**Negative**

Table 1. Allogeneic FCXM interpretation. A positive T cell crossmatch is defined as a delta MESF > 3,370 and a positive B cell crossmatch is defined as a delta MESF > 4,425 when using lymphocytes isolated from the Lymph node (at time of Tx).

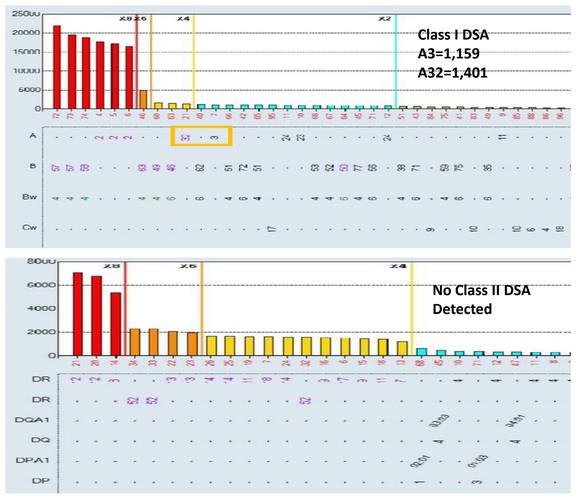

Figure 1. Single Antigen Beads Assay testing results at the time of transplant

Both pre-existing DSA and *de novo* DSA show a dramatic increase by day 14 post-tx

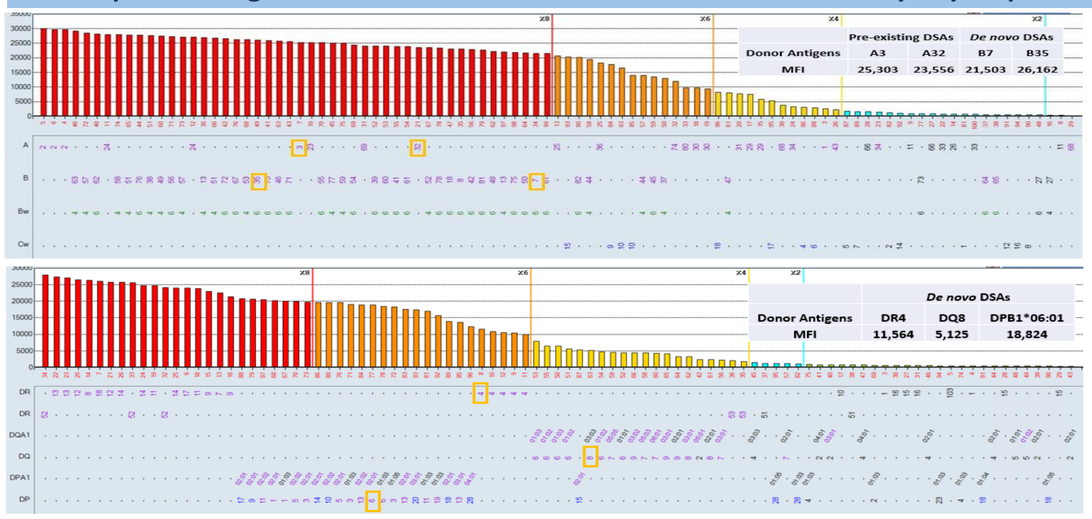

Figure 2. Single Antigen Beads Assay testing results at day 10 post-transplant

C1q testing and C4d Staining

C4d staining from the biopsy was positive at day 10 post-transplant
C1q studies of serum from day 16 revealed complement fixation for HLA-A3, -A32, -B7, and -B35

HLA Class I and II DSA MFI values initially decreased after plasmapheresis treatment but rebounded over time

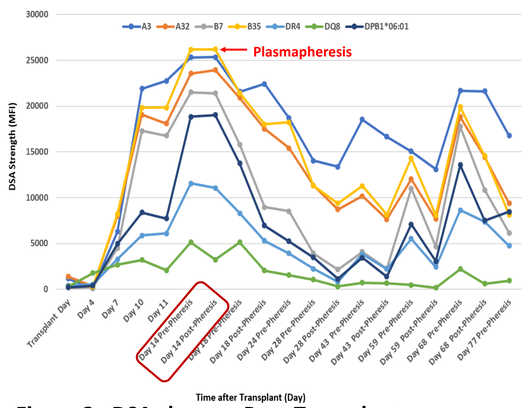

Figure 3 . DSA changes Post-Transplant

Eplet analysis revealed shared eplets (76E and 77S) between the *de novo* HLA-B7 and -B35 antigens and HLA-A32

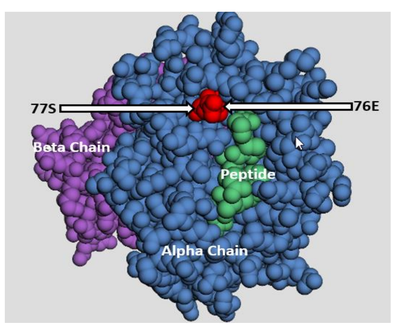

Figure 4 . Location of eplets 76E and 77S (red) in the peptide binding groove of the HLA-A32 molecule, top view (credit: pHLA3D).

PRINCIPAL FINDINGS

- Our study found that a pediatric patient with low level of circulating DSAs pre-transplant may experience a robust memory response as well as develop *de novo* DSAs directed at other mismatched donor HLA antigens very quickly in heart transplantation.
- The detection of complement-binding anti-HLA donor-specific antibodies post-transplantation is strongly associated with graft injury and often graft loss and should be monitored closely once detected.
- Eplet analysis may help interpret *de novo* DSA development and the response to plasmapheresis differently in Class I and Class II antibodies.

IMPLICATIONS FOR CLINICAL PRACTICE

- Patients harboring both pre-existing and post-transplant *de novo* DSA may experience notably elevated rates of AMR and comparatively diminished graft survival in contrast to those lacking DSA.
- The assessment of risk for memory or primary alloimmune response is very critical for transplant outcome.

CONCLUSIONS

Pre-existing DSAs with initially low MFI values can greatly impact both organ and patient survival in heart transplantation, and should be monitored closely.

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