

# Synergistic effect of DSA and non-HLA antibodies on T cellmediated rejection of adult liver allografts

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# Introduction

Donor-specific HLA antibodies (DSA) are associated with rejecting kidney, heart, and lung transplants. However, the impact of DSA is controversial in the liver transplant literature. Here, we postulate that multiple insults, including DSA and non-HLA antibodies, contribute to injuries of liver allografts. We aim to examine the roles of these antibodies in T-cell mediated rejection (TCMR) of adult liver transplants.

# Methods and Materials

- Inclusion criteria: 1) Adult liver transplant recipients at UPMC (2014 -2023); 2) liver biopsies with a serum tested for DSA within a month of biopsy date; 2) available serums for retrospective testing of non-HLA antibodies.
- 103 biopsy-serum pairs are included. Biopsies were done with a median of 366 (IQR:171 -732) days post-transplant, a mean of  $2 \pm 9$  days before serum collection.

## **Table 2**. Antibodies associated with Moderate-Severe TCMR

|  | TCMR: Neg-Mild (n=77) | TCMR: Mod-Severe<br>(n=26) | HR (95% CI)           | P- val  |
|--|-----------------------|----------------------------|-----------------------|---------|
| DSA MFI > 1 k                                  |                       |                            |                       |         |
| Negative                                       | 5                     | 3                          | 1                     |         |
| Positive                                       | 72                    | 23                         | 0.53 (0.12 -2.40)     | 0.412   |
| DSA MFI > 5 k                                  |                       |                            |                       |         |
| Negative                                       | 31                    | 8                          | 1                     |         |
| Positive                                       | 46                    | 18                         | 1.28 (0.80 – 2.06)    | 0.302   |
| Anti-AT1R(U/ml), Median (IQR)                  | 13.4 (10.0 - 21.1)    | 18.8 (15.1 - 40.0)         | 1.05 (1.01 - 1.09)    | 0.012   |
| Anti-AT1R positivity, n                        |                       |                            |                       |         |
| Negative (<10U/ml)                             | 19                    | 1                          | 1                     |         |
| At risk (>10 <i>,</i> < 17 U/ml)               | 32                    | 8                          | 4.75 (0.55 - 40.98)   | 0.156   |
| Positive c)                                    | 26                    | 17                         | 12.42 (1.52 - 101.63) | 0.019   |
| Number of Panel-18 Non-HLA Antibodies,         |                       |                            |                       |         |
| Median (IQR)                                   | 1 (0 - 1)             | 3 (2 -5)                   | 3.83 (2.10 - 6.99)    | < 0.001 |
| Number of Panel-18 Non-HLA Antibodies, n       |                       |                            |                       |         |
| ≤ 2  | 72                    | 11                         | 1                     |         |
| ≥ 3  | 5                     | 15                         | 19.64 (5.95 - 64.84)  | < 0.001 |
| DSA (MFI > 5K), Panel-18 non-HLA Abs $\geq$ 3, |                       |                            |                       |         |
| AT1R Abs (> 17U/ml)                            |                       |                            |                       |         |
| All negative                                   | 21                    | 0                          |                       | < 0.001 |
| Single-positive                                | 36                    | 8                          |                       |         |
| Double-positive                                | 19                    | 12                         |                       |         |
| Triple-positive                                | 1                     | 6                          |                       |         |

Results

- Rejections were graded based on Banff criteria and categorized as Moderate-Severe TCMR (T cell-Mediate Rejection) and Negative-Mild TCMR. A total of 28 histological features were assessed to determine the mechanism of non-HLA Antibodies.
- Banked post-transplant sera were retrospectively tested with Luminex-based solid-phase assay for 60 non-HLA antibodies (LIFECODES Non-HLA Antibody, Immucor, Norcross, GA). Mean fluorescence intensity (MFI) was used for data analysis. Positive/negative reactions were determined based on Vendor-provided cutoffs. Chi-square tests were done to select the non-HLA antibodies that were significantly associated with moderatesevere TCMR (p < 0.05).
- Positive DSA was determined with MFI>1K or 5K in Luminex Single Antigen beads assay (One Lambda, West Hills, CA) as routine patient care.
- HR (hazard ratio) was calculated with calculated with Binominal Logistic regression

|         | TCMR-NEG /  | TCMR-MOD /   | P-val  | P-val  | Antigen Detail                 | Anti-AT1R, >17U/ml |     |                      |         |  |
|---------|-------------|--------------|--|--|--------------------------------|--------------------|-----|----------------------|---------|--|
| Antigen | MILD (N=77) | SEVERE(N=26) |  |  |                                | Negative           | 51  | 9                    | 1       |  |
| AGRIN   | 0           | 3            | 0.002  | Agrin  | Positive                       | 29                 | 14  | 2 74 (1 05 -7 10)    | 0 039   |  |
| ARHGDIB | 10          | 9            | 0.014  | Rho GDP dissociation inhibitor beta                  | Number of Non III A Antibodies |                    | ± . |                      | 0.000   |  |
| ATP5B   | 5           | 6            | 0.018  | ATP synthase, beta polypeptide                       | Number of Non-FILA Antibodies  |                    |     |                      |         |  |
| CD40    | 0           | 3            | 0.002  | CD40 molecule, TNF receptor superfamily member 5     | ≤ 2                            | 74                 | 9   |                      |         |  |
| CXCR9   | 1           | 3            | 0.019  | C-X-C Motif Chemokine 9                              | ≥ 3                            | 6                  | 14  | 19.19 (5.89 – 62.26) | < 0.001 |  |
| DEXI    | 1           | 5            | < 0.001  | Dexamethasone-induced transcript                     | Anti-ARHGDIB                   |                    |     |                      |         |  |
| ENO1    | 22          | 16           | 0.003  | Alpha-enolase  | Negative                       | 69                 | 15  | 1                    |         |  |
| FLRT2   | 1           | 5            | < 0.001  | Leucine-rich repeat transmembrane protein FLRT2      | Positive                       | 11                 | 8   | 335(115-974)         | 0 027   |  |
| GSTT1   | 9           | 8            | 0.023  | Glutathione S- Transferase theta-1                   |                                |                    | U   | 5.55 (1.15 5.74)     | 0.027   |  |
| HARS    | 1           | 3            | 0.019  | Jo-1   | Anti-a-Enolase                 |                    |     |                      |         |  |
| LGALS3  | 2           | 4            | 0.016  | Lectin, galactoside- binding, soluble, 3             | Negative                       | 55                 | 10  | 1                    |         |  |
| NCL     | 2           | 6            | < 0.001  | Nucleolin  | Positive                       | 25                 | 13  | 2.86 (1.11-7.40)     | 0.030   |  |
| P2RY11  | 0           | 4            | < 0.001  | Purinergic receptor P2Y, G- protein coupled, 11      | Anti-GSTT1                     |                    |     |                      |         |  |
| PLA2R1  | 0           | 2            | 0.014  | Phospholipase A2 receptor 1, 180kDa                  | Negative                       | 71                 | 15  | 1                    |         |  |
| PTPRO   | 0           | 4            | < 0.001  | Receptor-type Tyrosine-protein Phosphatase U         | Positive                       | Q                  | 8   | 4 21 (1 40-12 68)    | 0 011   |  |
| SHC3    | 5           | 7            | 0.005  | SHC Adaptor Protein 3                                |                                |                    | U   | 4.21 (1.40 12.00)    | 0.011   |  |
| SNRPN   | Δ           | F            | 5 0.028 Small Nuclear Ribonucleoproteir<br>antigen cor | Small Nuclear Ribonucleoprotein Polypeptide N (smith | ANU-STAL 0                     | — -                |     |                      |         |  |
|         | 4           | 5            |  | antigen core)  | Negative                       | /4                 | 16  | 1                    |         |  |
| STAT6   | 6           | 7            | 0.011  | Signal Transducer And Activator Of Transcription 6   | Positive                       | 6                  | 7   | 5.40 (1.60 - 18.22)  | 0.007   |  |

### **Table 3**. Non-HLA antibodies are associated with Moderate-Severe Microvascular Injury (MVI)

| Table 1.  | Panel of 18 I | non-HLA antibo                 | dies are                             | e significantly associated with                      |                              | MVI: None/Minimal/Mild | <b>MVI: Moderate/Severe</b> |                      |         |
|---|---------------|--------------------------------|--------------------------------------|--|------------------------------|------------------------|-----------------------------|----------------------|---------|
| Moderate/Severe TCMR. The ones with > 10 incidences were highlighted in red |               |                                | 0 incidences were highlighted in red |  | (n=80)                       | (n=23)                 | HR (95 % CI)                | P-val                |         |
| TCMR-NE   | TCMR-NEG /    | / TCMR-MOD /<br>) SEVERE(N=26) | P-val                                | Antigen Detail                                       | Anti-AT1R, >17U/ml           |                        |                             |                      |         |
| Antigen   | MILD (N=77)   |                                |                                      |  |                              |                        | Negative                    | 51                   | 9       |
| AGRIN   | 0             | 3                              | 0.002                                | Agrin  | Positive                     | 29                     | 14                          | 2.74 (1.05 -7.10)    | 0.039   |
| ARHGDIB   | 10            | 9                              | 0.014                                | Rho GDP dissociation inhibitor beta                  | Number of Non-HLA Antibodies |                        |                             |                      |         |
| ATP5B   | 5             | 6                              | 0.018                                | ATP synthase, beta polypeptide                       |                              | 71                     | 0                           | N N                  |         |
| CD40  | 0             | 3                              | 0.002                                | CD40 molecule, TNF receptor superfamily member 5     | $\leq Z$                     | /4                     | 9                           |                      |         |
| CXCR9   | 1             | 3                              | 0.019                                | C-X-C Motif Chemokine 9                              | ≥ 3                          | 6                      | 14                          | 19.19 (5.89 – 62.26) | < 0.001 |
| DEXI  | 1             | 5                              | < 0.001                              | Dexamethasone-induced transcript                     | Anti-ARHGDIB                 |                        |                             |                      |         |
| ENO1  | 22            | 16                             | 0.003                                | Alpha-enolase  | Negative                     | 69                     | 15                          | 1                    |         |
| FLRT2   | 1             | 5                              | < 0.001                              | Leucine-rich repeat transmembrane protein FLRT2      | Positive                     | 11                     | 8                           | 335(115 - 974)       | 0 027   |
| GSTT1   | 9             | 8                              | 0.023                                | Glutathione S- Transferase theta-1                   | Anti a Englaco               |                        | 0                           | 5.55 (1.15 5.74)     | 0.027   |
| HARS  | 1             | 3                              | 0.019                                | Jo-1   | Anti-α-Enolase               |                        |                             |                      |         |
| LGALS3  | 2             | 4                              | 0.016                                | Lectin, galactoside- binding, soluble, 3             | Negative                     | 55                     | 10                          | 1                    |         |
| NCL   | 2             | 6                              | < 0.001                              | Nucleolin  | Positive                     | 25                     | 13                          | 2.86 (1.11-7.40)     | 0.030   |
| P2RY11  | 0             | 4                              | < 0.001                              | Purinergic receptor P2Y, G- protein coupled, 11      | Anti-GSTT1                   |                        |                             |                      |         |
| PLA2R1  | 0             | 2                              | 0.014                                | Phospholipase A2 receptor 1, 180kDa                  | Negative                     | 71                     | 15                          | 1                    |         |
| PTPRO   | 0             | 4                              | < 0.001                              | Receptor-type Tyrosine-protein Phosphatase U         | Positivo                     | 9                      | 8                           |                      | 0 011   |
| SHC3  | 5             | 7                              | 0.005                                | SHC Adaptor Protein 3                                |                              | 5                      | 0                           | 4.21 (1.40-12.08)    | 0.011   |
| SNRPN 4   |               | -                              | 5 0.028                              | Small Nuclear Ribonucleoprotein Polypeptide N (smith | ANTI-STAT 6                  |                        |                             |                      |         |
|   | 4             | 5                              |                                      | antigen core)  | Negative                     | 74                     | 16                          | 1                    |         |
| STAT6   | 6             | 7                              | 0.011                                | Signal Transducer And Activator Of Transcription 6   | Positive                     | 6                      | 7                           | 5.40 (1.60 - 18.22)  | 0.007   |

# Conclusions

- 1. In a cohort enriched for DSA-positive cases, DSA alone was not associated with increased moderate-severe TCMR risk.
- 2. Circulating antibodies to non-HLA antigens, such as AT1R or non-HLA panels, were associated with an augmented risk for moderate to severe TCMR and microvascular injury.
- 3. When DSA and non-HLA antibodies were detected concomitantly, the risk for moderate-severe TCMR was increased significantly.

# Discussion

Previously, we reported that pre-formed non-HLA antibodies to AT1R (1) and LG3 (laminin-like globular domain of Perlecan) (2) are strongly associated with poor graft survival in liver re-transplant recipients, while pre-formed DSA did not (3). However, posttransplantation DSA/non-HLA antibodies were not examined, nor was the histology of liver allograft biopsies. In the current study with detailed data, we found a synergistic effect of DSA and multiple non-HLA antibodies on moderate-severe TCMR. Interestingly, DSA alone was not associated with TCMR, possibly due to a lack of DSA-negative control cases in the cohort. Our findings confirm the previous reports in lung (4) and heart (5) that multiple insults from antibodies to HLA and non-HLA antigens may contribute to allograft injury. The possible mechanism of non-HLA antibodies in TCMR might be involved in Microvascular Injury (MVI) in liver allografts, confirming the previously reported anti-AT1R and MVI in renal allografts (6).





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