Confounding variables such as previous transplants, gender, ethnicity, and primary disease complicate the diagnostic validity of single-antigen bead-based non-HLA antibody assays

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Purpose

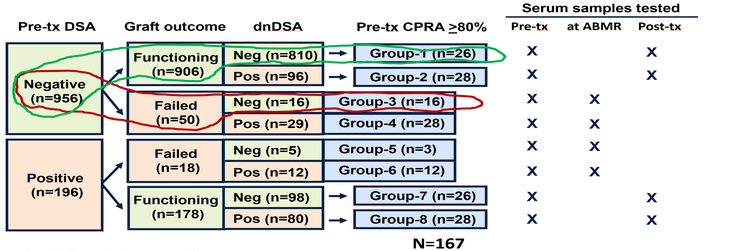
- Increasing evidence suggests a correlation between non-HLA antibodies and rejection of kidney transplants.
- Available non-HLA antibody assays detect one or limited non-HLA antibody specificities and are developed in research laboratories.
- We utilized a panel of 82 unique non-HLA conjugated Luminex bead assay (Werfen/Immucor) to determine non-HLA antibodies in kidney transplant outcomes.

Methods

- Based on pre-transplant HLA-DSA, de novo HLA-DSA, and kidney transplant outcomes, we have included 167 patients who received a deceased donor kidney transplant at UCSF from 2013 to 2018.
- All recipients with functioning grafts were highly sensitized with a cPRA of ≥80%.
- Sera collected within 3 months before kidney transplant and 6 months post-transplant were tested for non-HLA antibody using Luminex assay.

Study cohort design

Recipients of deceased donor kidney transplants performed at UCSF (Jan 2013-Dec 2018)



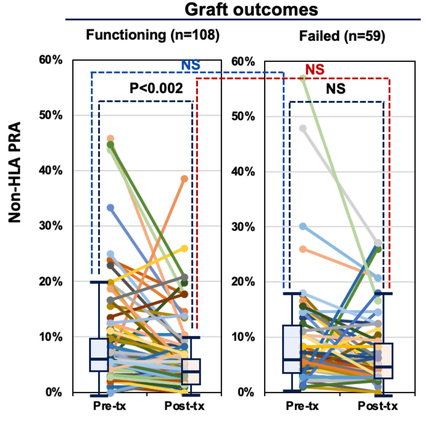
Immucor Non-HLA Panel Composition

Antigen	Gene Name
Actin	Actin
AGRN	Agrin
APOL2	apolipoprotein L, 2
ARHGDIB	Rho GDP-dissociation inhibitor 2
ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex
CCP	Cyclic citrullinated peptide
CD40	CD40 molecule, TNF receptor superfamily member 5
CGB5	chorionic gonadotropin, beta polypeptide 5
Collagen I	Collagen I
Collagen II	Collagen II
Collagen III	Collagen III
Collagen IV	Collagen IV
Collagen V	Collagen V
Collagen VI	Collagen VI
CSF2	Colony stimulating factor 2
CXCL11	chemokine (C-X- C motif) ligand 11
CXCL9	C-X-C Motif Chemokine 9
DEXI	dexamethasoneinduced transcript
EMCN	Endomucin
ENO1	Alpha-enolase
FAS	Fas cell surface death receptor
FN1	fibronectin 1
FLRT2	Leucine-rich repeat transmembrane protein FLRT2
GAPDH	Glyceraldehyde- 3-phosphate dehydrogenase
GDNF	glial cell derived neurotrophic factor
GSTT1	Glutathione S- Transferase theta-1
HARS	Jo-1
HSPB1	Heat shock protein beta-1
ICAM1	Intracellular Adhesion Molecule 1
IFNG	Interferon Gamma
IL21	Interleukin 21
IL8	Interleukin 8, CXCL8
KRT18	Cytokeratin 18
KRT8	Cytokeratin 8
LGALS3	Galectin 3
LGALS8	Galectin 8
LMNA	Prelamin-A/C
LPHN1	Latrophilin 1
Myosin	Myosin
NCL	nucleolin
P2RY11	purinergic receptor P2Y, G- protein coupled, 11
PECR	Peroxisomal trans-2-enoyl- CoA Reductase
PLA2R1	phospholipase A2 receptor 1, 180kDa
PRKCH	Protein kinase C, eta
PRKCZ	protein kinase C, zeta
PTPRO	Receptor-type Tyrosine-protein Phosphatase U
ROR1	Receptor Tyrosine KinaseLike Orphan Receptor 1
SHC3	SHC-Transforming Protein 3
SNRPB2	small nuclear ribonucleoprotei n polypeptide B
SNRPN	Small Nuclear Ribonucleoprotein Polypeptide N (smith antigen core)
SSB	Sjogren syndrome antigen B (autoantigen La)
STAT6	Signal Transducer and Activator of Transcription 6, Interleukin-4 Induced
Thyroglobulin	Thyroglobulin
Transferrin	Transferrin
TUBA1B	tubulin, alpha 1b
TUBB	Tubulin
Tubulin	Tubulin
VCL	Vinculin
VEGFA	Vascular endothelial growth factor A
VIM	Vimentin

Results

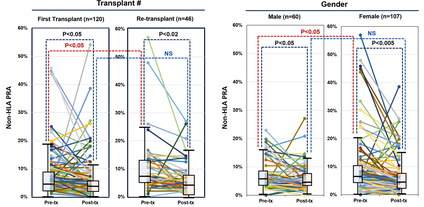
- Kidney transplant outcomes were not associated with either a specific or subset of non-HLA antibody.
- However, the non-HLA PRA values (calculated by the number of non-HLA beads positive divided by the number of non-HLA beads tested, multiplied by 100) remain unchanged in pre- and post-tx samples in recipients showing rejection (n=59).
- Non-HLA PRA values dropped significantly in post-transplant samples of those with functioning grafts (n=108) compared to pre-transplant samples (p<0.002).

Non-HLA antibodies are persistent in recipients showing rejection

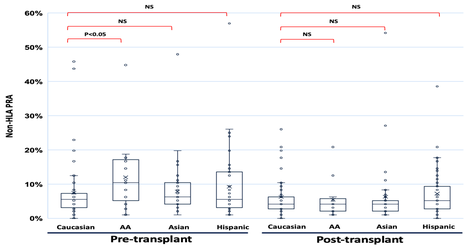


Results

- In cohort negative for both preformed and de novo HLA-DSA, those with graft failure maintained the non-HLA PRA same at pre-and post-transplant, while those with functioning graft dropped non-HLA PRA in post-transplant compared to pre-transplant (p<0.05).
- The pre-transplant non-HLA PRA are significantly elevated (p<0.05) in re-transplant recipients and female recipients compared to first-transplant and male recipients, respectively, indicating sensitization to allogenic tissues can trigger non-HLA antibody production.

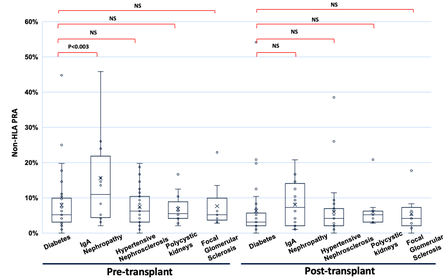


- African American patients exhibited higher pre-transplant non-HLA PRA compared to other populations



Results

- Those with IgA nephropathy as an etiology of ESRD had higher non-HLA PRA pre-transplant compared to other underlying conditions.



- There was no correlation between non-HLA antibody or PRA and endothelial crossmatch.

Conclusion

- Non-HLA antibodies significantly decreased in those with functioning grafts but remained elevated in those with graft loss.
- Since several confounding variables (previous transplant, gender, ethnicity, and primary disease) contribute to the non-HLA antibody production, further systematic multicenter studies with multivariate analyses are warranted to determine the clinical validity of non-HLA antibody testing.