SCREENING FOR NON-HLA ANTIBODIES - THE VERY FIRST RESULTS

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INTRODUCTION

Development of antibodies against non-HLA antigens (non-HLA antibodies) is likely associated with antibody mediated rejection (AMR) and has potential deleterious effects on graft survival in solid organ transplantation. Detection of such antibodies may additionally point to patients with increased immunologic risk for transplantation. The evidence for non-HLA antibodies causing AMR needs to be proofed with validated screening assays and verified results interpretation.

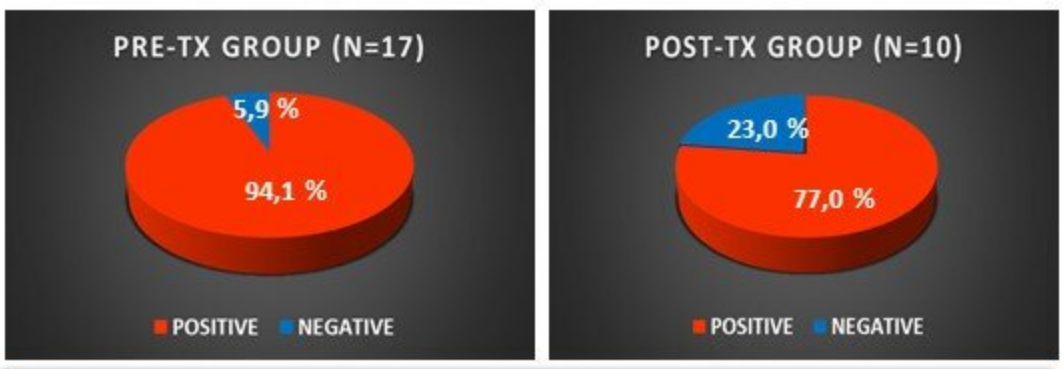
MATERIAL AND METHODS

We tested one of the two currently available non-HLA antibody assays based on Luminex platform which allows the direct simultaneous detection of IgG antibodies to 60 non-HLA auto-antigens (LIFECODES, Immucor) (Figure 1). Minimum two-fold median fluorescence intensity (MFI) value compared with test providers control was chosen as the positive threshold. The tested cohort consisted of 17 HLA highly sensitized recipients on the kidney waiting list (pre-tx group) and 10 kidney transplant recipients with biopsies showing evidence of AMR in the absence of detectable HLA DSA (post-tx group).

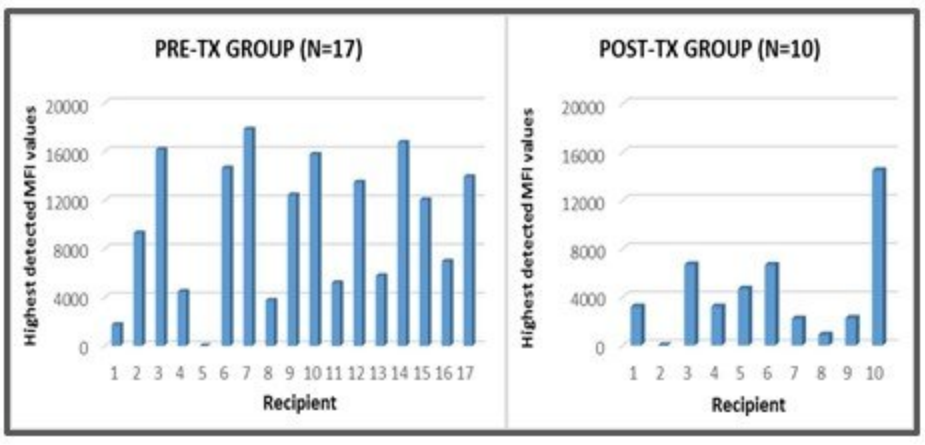
Antigen	Gene Name	Antigen	Gene Name
ACTIN	Actin	IFNG	Interferon Gamma
AGRN	Agrin	IL21	Interleukin 21
APOL2	Apolipoprotein L2	IL8	Interleukin 8, CXCL8
ARHGDIB	ARHGDIB	KRT18	Cytokeratin 18
ATP5B	ATP Synthase F1 Subunit Beta	KRT8	Cytokeratin 8
CCP	Cyclic Citrullinated Peptide	LGALS3	Galectin 3
CD40	CD40 molecule	LGALS8	Galectin 8
CGB5	Chorionic Gonadotropin Subunit Beta 5	LMNA	Lamin-A/C
COLLAGEN I	Collagen I	LPHN1	Latrophilin 1
COLLAGEN II	Collagen II	Myosin	Myosin
COLLAGEN III	Collagen III	NCL	Nucleolin
COLLAGEN IV	Collagen IV	P2RY11	Purinergic Receptor P2Y11
COLLAGEN V	Collagen V	PECR	Peroxisomal Trans-2-enoyl-CoA Reductase
COLLAGEN VI	Collagen VI	PLA2R1	Phospholipase A2 Receptor 1
CSF2	Colony Stimulating Factor 2	PRKCH	Protein kinase C, Eta
CXCL11	C-X-C Motif Chemokine Ligand 11	PRKCZ	Protein kinase C, Zeta
CXCL9	C-X-C Motif Chemokine Ligand 9	PTPRO	Receptor-type Tyrosine - protein Phosphatase U
DEXI	Dexamethasone-induced transcript	ROR1	Receptor Tyrosine Kinase-like Orphan Receptor 1
EMCN	Endomucin	SHC3	SHC Adaptor Protein 3
ENO1	Alpha-enolase	SNRPB2	Small Nuclear Ribonucleoprotein Polypeptide B
FAS	Fas Cell Surface Death Receptor	SNRPN	Small Nuclear Ribonucleoprotein Polypeptide N
FIBRONECTIN 1	Fibronectin 1	SSB	Sjogren Syndrome Antigen B
FLRT2	Fibronectin Leucine Rich Transmembrane Protein 2	STAT6	Signal Transducer And Activator of Transcription 6
GAPDH	Glyceraldehyde-3-phosphate Dehydrogenase	Thyroglobulin	Thyroglobulin
GDNF	Glial Cell Derived Neurotrophic Factor	TUBA1B	Tubulin Alpha 1b
GSTT1	Glutathione S-Transferase Theta-1	TUBB	Tubulin Beta
HARS	Jo-1	TUBULIN	Tubulin
HSPB1	Heat Shock Protein Beta-1	VCL	Vinculin
Human Transferrin	Transferrin	VEGFA	Vascular Endothelial Growth Factor A
ICAM1	Intracellular Adhesion Molecule 1	VIM	Vimentin

Figure 1: The LIFECODES panel of 60 non-HLA autoantigens

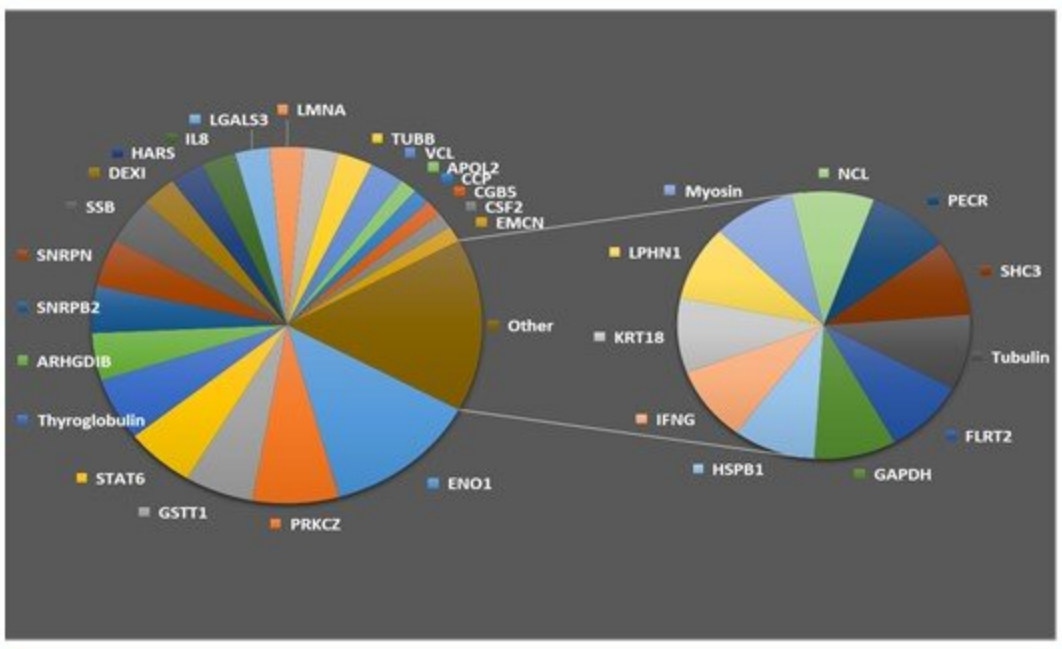
RESULTS

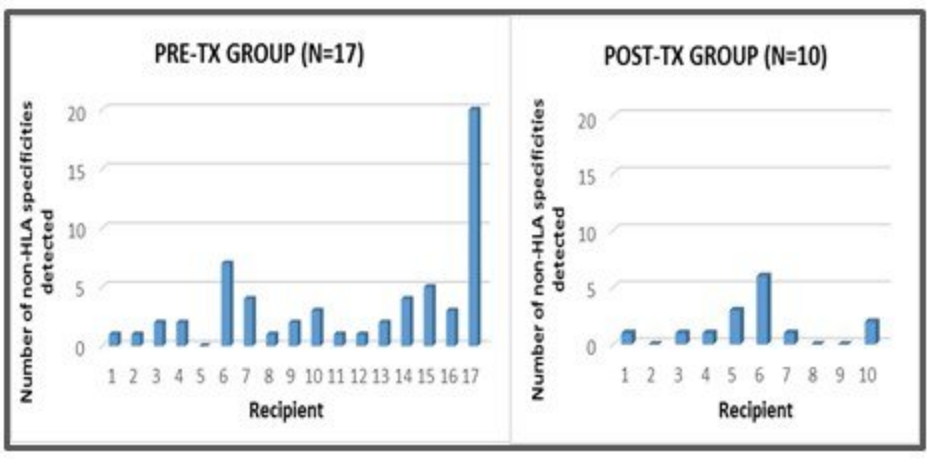


The presence of at least 1 of non-HLA antibody was detected in 16/17 (94.1%) pre-tx group recipients while in post-tx group non-HLA antibody was detected in 7/10 (77.0%) recipients.



The MFI values for positive reactions were much higher in pre-tx group (mean: 6229.7) comparing to post-tx group (mean: 4231.3).





In total, positive reactions against 33 different non-HLA antigens were observed. The most frequent reaction observed was against alpha-enolase (37.5%) and protein kinase C zeta (20.8%).

Number of different non-HLA specificities detected per recipient was much higher in pre-tx group (range: 1-22) than among post-tx recipients (range: 1-6).

CONCLUSION

The assay enabled highly sensitive detection and characterization of non-HLA antibodies.

Several conclusions can be drawn:

a) the presence of non-HLA antibodies is high in both investigated groups;
b) the number of specificity and intensity of positive reactions is significantly higher in HLA highly sensitized recipients;
c) suspicion of AMR without DSA can be associated with non-HLA antibodies in the post-tx group.

Although their role in transplantation is not yet completely clear, screening for non-HLA antibodies provides useful additional information which can help in identification of patients with an increased immunologic risk either prior to transplantation or post-transplantation.