The clinical relevance of non-HLA antibodies in antibody-mediated rejection in a single centre kidney transplant patient cohort

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BACKGROUND

It has been well established that anti-HLA donor-specific antibodies (DSAs) are the main cause of antibody-mediated rejection (AMR). There is anecdotal evidence, however, that non-HLA antibodies might be involved in AMR episodes after solid organ transplantation with an increasing number of patients presenting with pathological features of AMR in the absence of anti-HLA DSA[1]. Furthermore, AMR has been seen in recipients following transplantation from a HLA identical sibling[2]. Whilst there is evidence that AMR can be caused by non-HLA donor-specific antibodies, this area has not been investigated extensively and there is uncertainty surrounding the circumstances in which these antibodies are generated and the development of their pathogenic potential.

Serum samples from the study group (n = 23) were screened using LIFECODES® non-HLA antibody kits, which is a multiplex assay that allows the detection of antibodies against 60 non-HLA markers:

Antigen	Preferred Name	Antigen	Preferred 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	Prelamin-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, 180kDa
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
FIBRONECTIN1	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

Pre- and post-transplant serum samples were chosen to establish any changes in non-HLA antibody profiles following transplantation and whether non-HLA antibodies had a role in graft rejection in these patients.

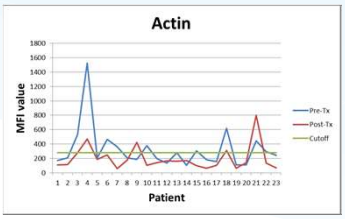
METHODS

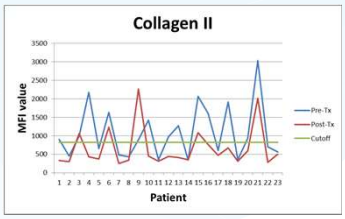
- 10µl patient serum added to 40µl non-HLA beads. Incubate at 22°C for 30 mins in the dark on a rotating platform
- 3 washes with 250µl wash buffer
- 50µl conjugate added (1 in 10 dilution). Incubate at 22°C for 30 mins in the dark on a rotating platform
- 150µl wash buffer added to each well and plate run on either Luminex® LABScan3D™ or Luminex® 200™ platform

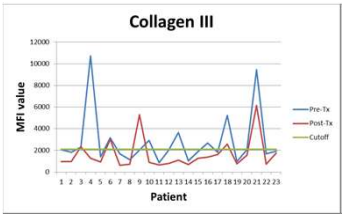
Assays are performed in 96 well filter plates. Supernatants were discarded using a vacuum manifold.

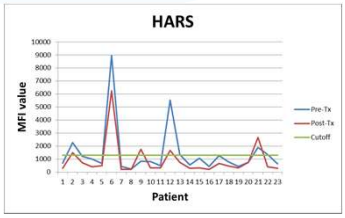
RESULTS

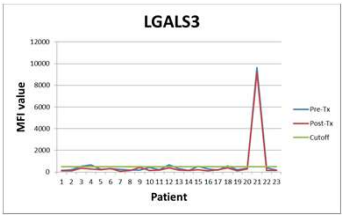
Of the 60 non-HLA markers, a significant difference ($p < 0.05$) in pre- and post-transplant mean fluorescence intensity (MFI) values was seen in 15 markers. Lower MFI values were seen post-transplant for all 15 statistically significant markers. Of these, 5 markers met the suggested cut-off value to be classed as positive – Actin ($p = 0.03$), Collagen II ($p = 0.004$), Collagen III ($p = 0.02$), HARS ($p = 0.02$) and LGALS3 ($p = 0.0006$).











DISCUSSION

A significant difference in pre- and post-transplant MFI values was seen in 25% of the non-HLA markers tested. Interestingly, significantly lower MFI values were seen post-transplant across all of these markers. However, it was expected that non-HLA antibody MFI values would have been higher post-transplant as this would suggest a potential role for these antibodies in patients with suspected AMR but no anti-HLA DSA. Lower non-HLA antibody MFI values post-transplant could be explained by the effects of immunosuppressive drugs given to patients following transplantation. All 23 patients had antibodies against at least one non-HLA antigen. This suggests that these antibodies might be autoimmune in nature.

CONCLUSIONS

Graft rejection is multifaceted and the potential effects of non-HLA antibodies in graft rejection are poorly understood. There also may be other factors that influence the pathogenicity of non-HLA antibodies on allografts. Further research is required in this area. In particular, larger, collaborative studies could aid in establishing the clinical relevance of non-HLA antibodies in solid organ transplantation. Further research could also include an investigation of the relevance of non-HLA antibodies in patients with AMR in the presence HLA DSA.

REFERENCES

1. Jordan SC. Immune response to non-HLA antigens and renal allograft loss. *The Lancet.* 2019;393(10174): 854-86.
2. Grafft CA, Cornell LD, Gloor JM, Cosio FG, Gandhi MJ, Dean PG, Stegall MD, Amer H. Antibody-mediated rejection following transplantation from an HLA-identical sibling. *Nephrology Dialysis Transplantation.* 2010;25(1): 307-310.