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INTRODUCTION

- In absence or presence of donor-specific anti-HLA antibodies, antibodies directed against autoantigens (non-HLA antibodies) were suggested to represent a potential risk factor in developing antibody-mediated rejection (AMR) in kidney transplantation.
- Luminex-based single antigen kits allowing detection of antibodies to various autoantigens have been developed by different vendors and are in use in some HLA laboratories.

AIM

- Compare reactivities to autoantigens represented in two commercially available non-HLA single antigen kits.
- Assess the potential association of reactivities against autoantigens common to both kits with AMR in kidney transplant recipients.

METHODS

Study design and case-control selection:

- Nested case-control study from a multicenter retrospective cohort of first-time kidney-only transplant recipients from 2008 to 2019.
- Cases: recipients with AMR across its continuum diagnosed according to the Banff classification.
- Controls: recipients randomly selected from the remaining cohort and matched on center, transplant year, donor type and time post-transplant.

Serum samples and autoantibody testing:

- Pre-transplant samples (day of transplant or preceding day, n=167) and post-transplant samples (in proximity to biopsy date, n=159) from 91 cases and 96 controls were tested. Both pre-transplant and post-transplant samples were available only for 37 pairs of cases and matched-controls.
- Samples were tested using Werfen Non-HLA and One Lambda Autoantibody Luminex-based single antigen bead kits, according to the manufacturers' instructions
- Reactivities were deemed positive based on the manufacturer's recommended MFI cut-offs (bead-corrected MFI for Werfen and 95% confidence interval (95% CI) for One Lambda).

Statistical analyses:

- Agreement between assays was estimated using Cohen's kappa statistics and reported as K estimates and 95% CI.
- Associations between positive reactivities to autoantigens and AMR for pre-transplant and post-transplant time points was evaluated using conditional logistic regression and reported as point estimates (odd ratio, OR) and 95% CI.

RESULTS

Table 1. Agreement between Werfen Non-HLA and One Lambda Autoantibody single antigen kit results

Autoantigen	Pre-transplant samples (n=167)		Post-transplant samples (n=159)	
	k estimate	95% CI	k estimate	95% CI
AGNR	0.11	-0.03, 0.25	0.16	0.07, 0.24
ARHGDIIB	0.32	0.17, 0.46	0.26	0.11, 0.42
COLLAGEN I	-0.04	-0.22, 0.10	0.06	-0.22, 0.10
COLLAGEN II	0.22	0.09, 0.34	0.07	-0.08, 0.21
COLLAGEN III	0.10	-0.02, 0.22	0.16	0.05, 0.27
COLLAGEN IV	0.21	0.06, 0.36	-0.02	-0.17, 0.13
COLLAGEN V	0.15	0.00, 0.30	0.25	0.09, 0.40
CXCL11	0.14	0.02, 0.25	0.06	-0.04, 0.16
CXCL9	0.13	-0.01, 0.27	0.07	-0.04, 0.18
FIBRONECTIN1	0.06	-0.05, 0.18	-0.01	-0.08, 0.05
FLRT2	-0.02	-0.17, 0.13	0.23	0.09, 0.37
GAPDH	0.10	-0.05, 0.25	0.10	-0.04, 0.25
GDNF	0.46	0.31, 0.61	0.48	0.33, 0.64
GSST1	0.43	0.29, 0.57	0.36	0.24, 0.48
IFNG	0.24	0.11, 0.37	0.30	0.17, 0.43
LMNA	0.11	0.02, 0.20	0.06	-0.04, 0.17
MYOSIN	0.18	0.10, 0.27	0.16	0.07, 0.24
NCL	-0.01	-0.07, 0.05	0.00	0.00, 0.00
PECR	-0.02	-0.16, 0.12	0.00	-0.15, 0.15
PLA2R1	0.40	0.25, 0.54	0.26	0.12, 0.40
PRKCH	0.31	0.18, 0.44	0.38	0.26, 0.51
PRKCZ	0.11	0.01, 0.20	-0.04	-0.12, 0.05
TUBA1B	0.08	-0.06, 0.22	0.01	-0.13, 0.15

Agreement levels based on kappa estimates: ≤ 0.39 , minimal; 0.40 - 0.59, weak; 0.60 - 0.79, moderate; ≥ 0.80 , strong; 95% CI, 95% confidence interval.
Autoantigens: AGRN, Agrin; ARHGDIIB, Rho GDP Dissociation Inhibitor β ; CXCL11, C-X-C Motif Chemokine Ligand 11; CXCL-9, C-X-C Motif Chemokine Ligand 9; FLRT2, Fibronectin Leucine-Rich Repeat Transmembrane Protein 2; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; GDNF, Glial Cell-Derived Neurotrophic Factor; GSST1, Glutathione S-Transferase θ -1; IFNG, Interferon- γ ; LMNA, Lamin A/C; NCL, Nucleolin; PECR, Peroxisomal trans-2-Enoyl-CoA Reductase; PLA2R1, Phospholipase A2 Receptor 1; PRKCH, Protein Kinase C, η ; PRKCZ, Protein Kinase C, ζ ; TUBA1B, Tubulin α 1b.

Table 2. Association between reactivity against autoantigens represented in both Werfen and One Lambda Luminex-based single antigen kits, and antibody-mediated rejection across its continuum using post-transplant or pre-transplant serum samples

Autoantigen	Werfen Non-HLA kit				One Lambda Autoantibody kit			
	Pre-transplant		Post-transplant		Pre-transplant		Post-transplant	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
AGNR	-	-	-	-	2.50 (0.49, 12.89)	0.27	0.67 (0.11, 3.99)	0.66
ARHGDIIB	1.00 (0.25, 4.00)	1.00	2.00 (0.37, 10.92)	0.42	5.00 (0.58, 42.80)	0.14	5.00 (0.58, 42.80)	0.14
COLLAGEN I	1.50 (0.25, 8.98)	0.66	0.20 (0.02, 1.71)	0.14	1.00 (0.06, 15.99)	1.00	-	-
COLLAGEN II	0.56 (0.19, 1.66)	0.29	0.43 (0.11, 1.66)	0.22	0.50 (0.09, 2.73)	0.42	-	-
COLLAGEN III	0.44 (0.14, 1.44)	0.18	0.43 (0.11, 1.66)	0.22	0.38 (0.10, 1.41)	0.15	0.50 (0.17, 1.46)	0.21
COLLAGEN IV	0.50 (0.15, 1.66)	0.26	0.38 (0.10, 1.41)	0.15	0.80 (0.21, 2.98)	0.74	1.33 (0.30, 8.96)	0.71
COLLAGEN V	0.75 (0.26, 2.16)	0.59	0.14 (0.02, 1.16)	0.07	1.50 (0.25, 8.98)	0.66	2.00 (0.37, 10.92)	0.42
CXCL11	-	-	-	-	1.57 (0.61, 4.05)	0.35	3.33 (0.92, 12.11)	0.06
CXCL9	-	-	1.00 (0.06, 15.99)	1.00	0.75 (0.17, 3.35)	0.71	0.33 (0.03, 3.20)	0.34
FIBRONECTIN1	-	-	-	-	0.50 (0.09, 2.73)	0.42	0.33 (0.03, 3.20)	0.34
FLRT2	0.50 (0.09, 2.73)	0.42	0.67 (0.11, 3.99)	0.66	0.50 (0.17, 1.46)	0.21	1.00 (0.32, 3.10)	1.00
GAPDH	0.80 (0.21, 2.98)	0.74	0.50 (0.05, 5.51)	0.57	0.17 (0.02, 1.38)	0.10	0.13 (0.02, 1.00)	0.05
GDNF	0.60 (0.14, 2.51)	0.48	0.25 (0.03, 2.24)	0.22	0.50 (0.09, 2.73)	0.42	0.50 (0.09, 2.73)	0.42
GSST1	0.60 (0.14, 2.51)	0.48	1.00 (0.25, 4.00)	1.00	1.33 (0.30, 5.96)	0.71	1.33 (0.30, 5.96)	0.71
IFNG	1.33 (0.30, 5.96)	0.71	1.33 (0.30, 5.96)	0.71	0.78 (0.29, 2.09)	0.62	1.57 (0.61, 4.05)	0.35
LMNA	0.33 (0.07, 1.65)	0.18	0.25 (0.03, 2.24)	0.22	-	-	-	-
MYOSIN	0.43 (0.11, 1.66)	0.22	-	-	-	-	-	-
NCL	0.75 (0.17, 3.35)	0.71	3.00 (0.31, 28.84)	0.34	-	-	-	-
PECR	1.00 (0.25, 4.00)	1.00	1.00 (0.20, 4.95)	1.00	-	-	2.00 (0.18, 22.06)	0.57
PLA2R1	0.71 (0.23, 2.25)	0.56	0.13 (0.02, 1.00)	0.05	0.33 (0.07, 1.65)	0.18	0.33 (0.03, 3.20)	0.34
PRKCH	0.33 (0.09, 1.23)	0.10	1.33 (0.30, 5.96)	0.71	0.89 (0.34, 2.30)	0.81	0.80 (0.32, 2.03)	0.64
PRKCZ	0.71 (0.23, 2.25)	0.56	0.50 (0.15, 1.66)	0.26	1.00 (0.06, 15.99)	1.00	-	-
TUBA1B	0.50 (0.05, 5.51)	0.57	0.50 (0.05, 5.51)	0.57	6.00 (0.72, 49.84)	0.10	-	-

OR, Odds Ratio; 95% CI, 95% confidence interval; a p value ≤ 0.05 is considered statistically significant; "-", no estimates could be provided as there was no evidence of reactivity.

MAIN FINDINGS

- When assessing reactivity against autoantigens represented in both Werfen Non-HLA and One Lambda Autoantibody Luminex-based single antigen bead kits, we observed only weak agreement on three (GDNF, GSST1, PLA2R1) of the 23 shared autoantigens.
- Studying a subset of 37 matched case:control pairs, and accounting for multiple testing, there remains uncertainty as to the association of reactivity against autoantigens measured post-transplant or pre-transplant and AMR.

CONCLUSION

Our study demonstrates limited agreement on non-HLA antibody assignment between two commercially available Luminex-based single antigen bead assays. Given a growing interest in testing for non-HLA antibodies to assign AMR diagnosis by the Banff classification, further evaluation of assay performance, temporal changes in assay results post-transplant, and association with transplant outcomes in larger studies, are needed.

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