

Getting to Grips with Non-HLA antibody

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Werfen User Group Meeting Oct 25

Non-HLA/Auto antibody:

- Main focus for H&I labs when assessing compatibility are HLA specific antibodies.
- Preformed and de novo donor HLA specific antibodies are associated with poorer outcomes.
- However – up to 50% of AMR diagnosed on biopsy is not associated with the presence of circulating HLA donor specific antibody.
- The recently updated UK BSHI/BTS guidelines on the detection of alloantibodies in solid organ transplantation includes sections on the relevance of non-HLA antibodies in the different organ settings.
- The current version of the BANFF classification for renal biopsies allows the definition of antibody mediated rejection (microvascular inflammation) without HLA-DSA and suggests testing for non-HLA antibodies in these cases.
- Increasing numbers of publications around a variety of non-HLA targets and potential impact on renal transplant outcomes.

Non-HLA/Auto antibody:

- Non-HLA antibody can be –
 - an autoantibody or alloantibody,
 - detectable pre-transplant and/or de novo.
- The production of non-HLA autoantibodies can occur in a response to a loss of self-tolerance or after tissue damage resulting from ischaemia reperfusion injury (IRI), calcineurin toxicity, alloimmunity and/or chronic inflammation, resulting in the creation of cryptic antigens that become exposed to the immune system.
- Alternatively, where there is genetic disparity between donor and recipient, non-HLA alloantibodies may be produced.
- Currently the only non-HLA antibody that is routinely considered during an immunological risk assessment of recipient and donor is ABO compatibility (+/- HNA in UK).
- The main reasons for this are the ability to detect the antibody and knowledge of the relevance.

Non-HLA/Auto Antibody:

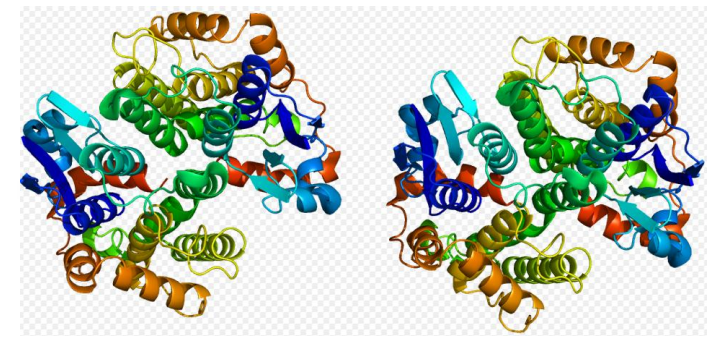
- Multiple studies identifying potential targets on endothelial cells in transplanted kidney allografts with independent associations with allograft injury.
- The most extensively studied non-HLA antibodies include –
 - Angiotensin II type 1 receptor (AT₁R),
 - MHC class I-related chain A (MICA)
 - Glutathione S-transferase theta-1 (GSTT).
- Some studies have found a synergistic effect of HLA and non-HLA antibody, but others have found independent associations.
- The impact the role of non-HLA antibodies is not limited to AMR, with implications in vascular rejection also identified.
- Non-HLA or Auto antibody kits are now available with multiple non-HLA targets for analysis.

Antigen	Gene Name	Exclusive to Immucor Luminex® Assay
ACTIN	Actin	✓
AGRN	Agrin	
APOL2	Apolipoprotein L2	✓
ARHGDIB	ARHGDIB	
ATP5B	ATP Synthase F1 Subunit Beta	✓
CCP	Cyclic Citrullinated Peptide	✓
CD40	CD40 molecule	✓
CGB5	Chorionic Gonadotropin Subunit Beta 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	C-X-C Motif Chemokine Ligand 11
CXCL9	C-X-C Motif Chemokine Ligand 9
DEXI	Dexamethasone-induced transcript
EMCN	Endomucin
ENO1	Alpha-enolase
FAS	Fas Cell Surface Death Receptor
FIBRONECTIN1	Fibronectin 1
FLRT2	Fibronectin Leucine Rich Transmembrane Protein 2
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
Human Transferrin	Transferrin
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	Lamin-A/C	
LPHN1	Latrophilin 1	✓
MYOSIN	Myosin	
NCL	Nucleolin	
P2RY11	Purinergic Receptor P2Y11	✓
PECR	Peroxisomal Trans-2-enoyl-CoA Reductase	
PLA2R1	Phospholipase A2 Receptor 1	
PRKCH	Protein kinase C, Eta	
PRKCZ	Protein kinase C, Zeta	
PTPRO	Receptor-type Tyrosine-protein Phosphatase U	✓
ROR1	Receptor Tyrosine Kinase- Like Orphan Receptor 1	✓
SHC3	SHC Adaptor Protein 3	✓
SNRNP2	Small Nuclear Ribonucleoprotein Polypeptide B	✓
SNRPN	Small Nuclear Ribonucleoprotein Polypeptide N	✓
SSB	Sjogren Syndrome Antigen B	✓
STAT6	Signal Transducer And Activator Of Transcription 6	✓
Thyroglobulin	Thyroglobulin	✓
TUBA1B	Tubulin Alpha 1b	
TUBB	Tubulin Beta	✓
TUBULIN	Tubulin	✓
VCL	Vinculin	✓
VEGFA	Vascular Endothelial Growth Factor A	✓
VIM	Vimentin	

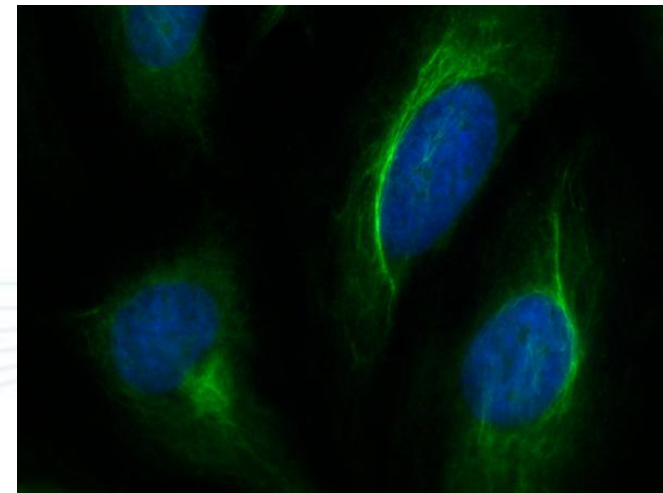
Anti-glutathione S-transferase theta-1 (GSTT1) antibodies



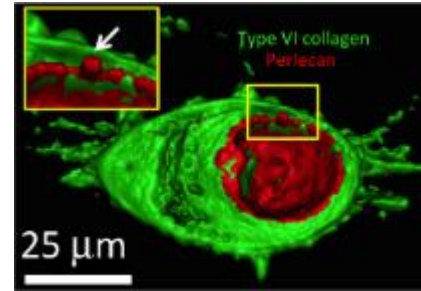
- Glutathione S-transferase theta-1 (GSTT1) protein is a member of an enzyme superfamily, expressed in the liver, kidney, pancreas, red blood cells and other tissues.
- Antibodies directed to GSTT1 can be either auto- or alloantibody.
- The protein is encoded by a single PM gene that has two alleles: GSTT1*A (positive) and GSTT1*0 (null).
- GSTT1 null recipients receiving a GSTT1 positive kidney have the potential to develop GSTT1-specific antibodies – with studies linking this to AMR.
- GSTT1 is present in around 20% of the Caucasian population and 11-58% of other ethnic groups
- Alternatively, as GSTT1 is not typically expressed on the cell surface, GSTT1 positive patients that receive a GSTT1 positive kidney, can produce autoantibodies to GSTT1 as a result of antigen recognition in the extracellular compartment caused by cell damage

Anti-vimentin

- Vimentin is an intracellular protein which polymerises to form the basis of the cytoskeleton in fibroblasts, podocytes, renal tubular, endothelial, smooth muscle, mesangial and renal stromal cells and is involved in the maintenance of the cellular organisation and structure and contribution to cell signalling and proliferation (Soroohan et al., 2022).
- Usually, vimentin is not expressed on the cell surface and its exposure can lead to antigen expression and the development of vimentin specific autoantibodies.
- The expression of vimentin on cell membranes and/or secreted into the serum can occur as a result of pro-inflammatory states such as apoptosis or endothelial damage caused by IRI, infection and rejection after transplant.



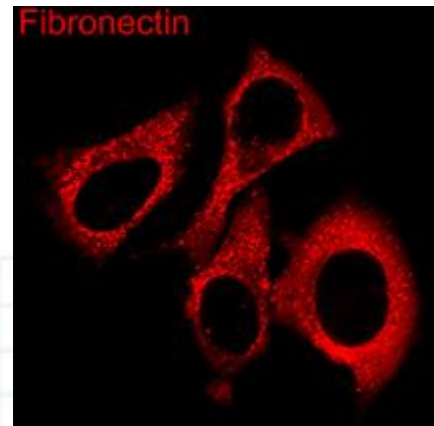
Anti-perlecan/LG3 antibodies



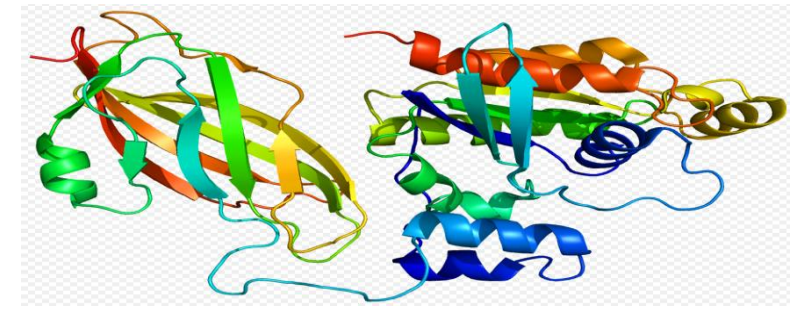
- Perlecan is a heparan sulphate proteoglycan (HSP) present in vascular and epithelial basement membranes, of which its C-terminal fragment, laminin-like globular domain (LG3) is highly immunogenic.
- Studies have found increased levels of LG3 in serum and urine in patients with acute and chronic vascular rejection respectively.
- LG3 can be released during apoptosis, resulting in the development of anti-LG3 antibodies.
- Anti-LG3 antibodies have been identified as novel accelerators of immune mediated vascular injury due to an increase in endothelial cell apoptosis
- It has been suggested that anti-LG3 antibodies can be novel aggravating factors for IRI, with passive transfer of anti-LG3 antibodies enhancing renal dysfunction due to IRI.

Anti-collagen and fibronectin antibodies

- Collagen and fibronectin are constituents of the glomerular basement membrane (GBM), of which duplication of the GBM is associated with chronic renal allograft rejection.
- Park et al., (2022) suggests a potential diagnostic and prognostic values of measuring anti-collagen antibody levels as it was found that a higher level of anti-collagen type I or III antibody was associated with a higher risk of death censored graft failure.
- However, Hesemann et al., (2015) found that de novo anti-collagen IV and anti-fibronectin antibodies in a paediatric population were not found to correlate with graft function or worse clinical outcomes.



Anti-Rho GDP-dissociation inhibitor 2 (ARHGDIB) antibodies



- The ARHGDIB gene encodes for the protein RhoGDI2 - expressed in the cytoplasm of kidney endothelial cells and podocytes, and on the cell surface of apoptotic cells.
- Kamurova et al., (2018) found a significant association with graft loss in patients who had pre-formed anti-ARHGDIB antibodies and received a deceased donor kidney, but no association was found in those who received a living donor kidney.
- Senev et al., (2020) found that pre-formed anti-ARHGDIB antibodies had an additive effect in patients with concurrent HLA-DQA, as their presence increased the risk of graft failure after AMR by 19.5-fold compared to patients who were negative for both.
- Currently no research has investigated the impact of *de novo* anti-ARHGDIB antibodies on AMR and graft failure.

Other non-HLA antibodies

- There are a number of studies that have found associations between the presence of singular non-HLA antibodies pre- and post-transplant and AMR. Notably,
- Senev et al., (2022) found patients who were broadly sensitised pre-transplant and with the highest total strength were independently associated with increased rates of histology of AMR post-transplant, in the absence of HLA-DSA.
- Four non-HLA antibodies (TUBB, Collagen III, VCL, STAT-6) were identified to be associated independently with AMR histology but overall the total pre-transplant burden was more informative.
- Bhutani et al., (2024) found that pre-transplant non-HLA antibodies GSTT, CXCL11, CXCL10 and HNR were associated with rejection.

Current Routine Non-HLA/Auto antibody assessment:

- Auto-antibodies may lead to 'false positive' crossmatches, but can often be excluded by methods such as performing an autologous crossmatch
- Cases of positive allo crossmatches with negative auto crossmatches in patients with no HLA antibodies (or no detected DSA) - investigation includes checks for patient medication (biologics for example), HNA-3a/b specific antibodies, recent illness, medical interventions or vaccines in the patient or donor.
- The more difficult to manage scenario is where patients with a negative crossmatch and no HLA DSA go on to have antibody mediated rejection (with or without C4d positivity on biopsy). This unexplained phenomena previously had little route for investigation by the H&I lab, other than confirming the negative HLA antibody test results.

Luminex based Non-HLA Kits

- General growing interest in 'Non-HLA' targets and antibodies – be it auto or allo.
- The recently updated UK BSHI/BTS guidelines on the detection of alloantibodies in solid organ transplantation includes sections on the relevance of non-HLA antibodies in the different organ settings.
- With this comes the increasing requests – 'What about the non-HLA?' – from the clinical team
- So far we aren't really able to answer this question with any certainty.
- We can test, but the reports come with the caveat that we don't really know what the results mean.

LIFECODES® Non-HLA Antibody Kit

SEE BEYOND THE LIMITS OF YOUR EXISTING WORKFLOW AND EXPAND
YOUR TEST CAPABILITIES WITH IMMUCOR'S NON-HLA ANTIBODY KIT.

How have we used the Werfen kit so far?

- To date we have limited experience with the non-HLA kit.
- Participated in the 18th International HLA & Immunogenetics Workshop project: Testing the clinical utility of commercial Non-HLA antibody kits
- Clinical testing has mainly been limited to specific patient cases – three examples -
 - Cases one & two – kits used to try to determine the cause of AMR seen on biopsy
 - Case three – kits used to reassure medical team in patient with previous non-HLA hyperacute rejection

Case 1 – AMR in HLA compatible tx

- Paediatric patient, aged 9, with congenital renal dysplasia
- Worked up for a transplant with either parent as the donor
- Crossmatched against both in 2021, XMs were negative
- All HLA antibody screening was negative, tested by One Lambda LabScreen multi – which includes HNA (also negative) and LIFECODES single antigen.
- Father selected as the donor, final crossmatch in July 2023, negative as before and still no HLA antibody detected. Tests now included One Lambda single antigen.
- Transplanted August 2023

Post Transplant

- A few days after the transplant, the patient's creatinine began to increase
- Further samples for HLA antibody testing were sent to the lab and a biopsy was performed
- HLA, HNA and MICA antibody testing still negative
- Biopsy showed slightly unusual humoral rejection pattern with inflammation of the inner lining of the arteries, but was also C4d negative
- The patient was given intravenous steroids and IVIg
- On day +8 a post transplant XM was performed, still negative
- Lab was asked if there was any further investigations we could do?

- Initial thoughts were the potential for an atypical red cell antibody, there have been cases described in the literature

Kidney Transplantation



OPEN

Hyperacute Antibody-mediated Rejection Associated With Red Blood Cell Antibodies

James Shaw, MD, PhD,¹ Ian W. Gibson, MD,² Chris Wiebe, MD,¹ Donald S. Houston, MD,³ Joshua Koulack, MD,⁴ David Rush, MD,¹ Peter Nickerson, MD,^{1,5} and Julie Ho, MD^{1,5}

- This was excluded, as the patient had negative screening reported from the blood transfusion laboratory

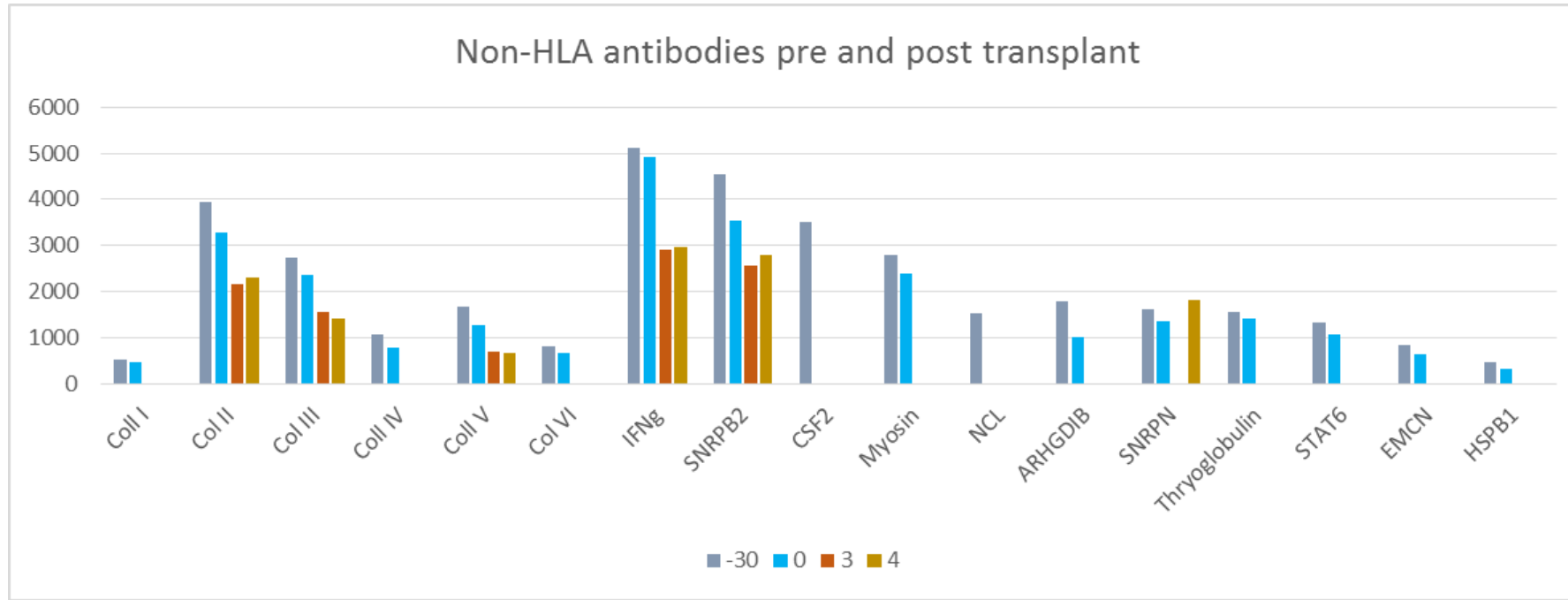
- We also tested some of the patient samples on the non-HLA antibody kit
- Samples from 1 month pre-tx, day of transplant, +3 and +4 days post transplant were tested.
- On the pre-tx samples, 18 of the 60 targets were positive. Seven of these remained positive in the post transplant samples.

Method Type: Standard Calculation

Positive CON MFI: 18652
Negative CON MFI: 133

Analysis Mode:
Manual

Bead	Bead Count	Raw Value	BCM	Background	Cutoff	Assignment	Antigen
533	58	5229	5121	108	216	Positive	IFNG
530	80	5102	4538	564	1128	Positive	SNRPB2
517	209	4355	3926	429	858	Positive	COLLAGEN II
506	54	3628	3494	134	268	Positive	IL21
521	57	3613	3497	116	232	Positive	CSF2
514	260	3540	2781	759	1518	Positive	MYOSIN
518	181	3310	2729	581	1162	Positive	COLLAGEN III
540	40	2244	1530	714	1428	Positive	NCL
554	70	2221	1788	433	866	Positive	ARHGDI8
502	57	2174	1629	545	1090	Positive	SNRPN
520	237	2003	1673	330	660	Positive	COLLAGEN V
558	109	1989	1563	426	852	Positive	Thyroglobulin
524	64	1623	1343	280	560	Positive	STAT6
519	155	1309	1081	228	456	Positive	COLLAGEN IV
557	87	1128	806	322	644	Positive	COLLAGEN VI
504	64	1010	834	176	352	Positive	EMCN
516	175	737	539	198	396	Positive	COLLAGEN I
545	63	597	465	132	264	Positive	HSPB1
544	116	3461	1534	1927	3854	Negative	FIBRONECTIN1
553	75	1778	90	1688	3376	Negative	VCL
505	60	1179	611	568	1136	Negative	SSB
525	62	947	374	573	1146	Negative	AGRN
512	79	904	564	340	680	Negative	KRT18
547	65	896	259	637	1274	Negative	HARS
541	66	727	268	459	918	Negative	PRKCH
538	63	685	263	422	844	Negative	LMNA
509	82	684	440	244	488	Negative	VIM
536	61	672	-132	804	1608	Negative	ENO1
523	72	671	269	402	804	Negative	SHC3

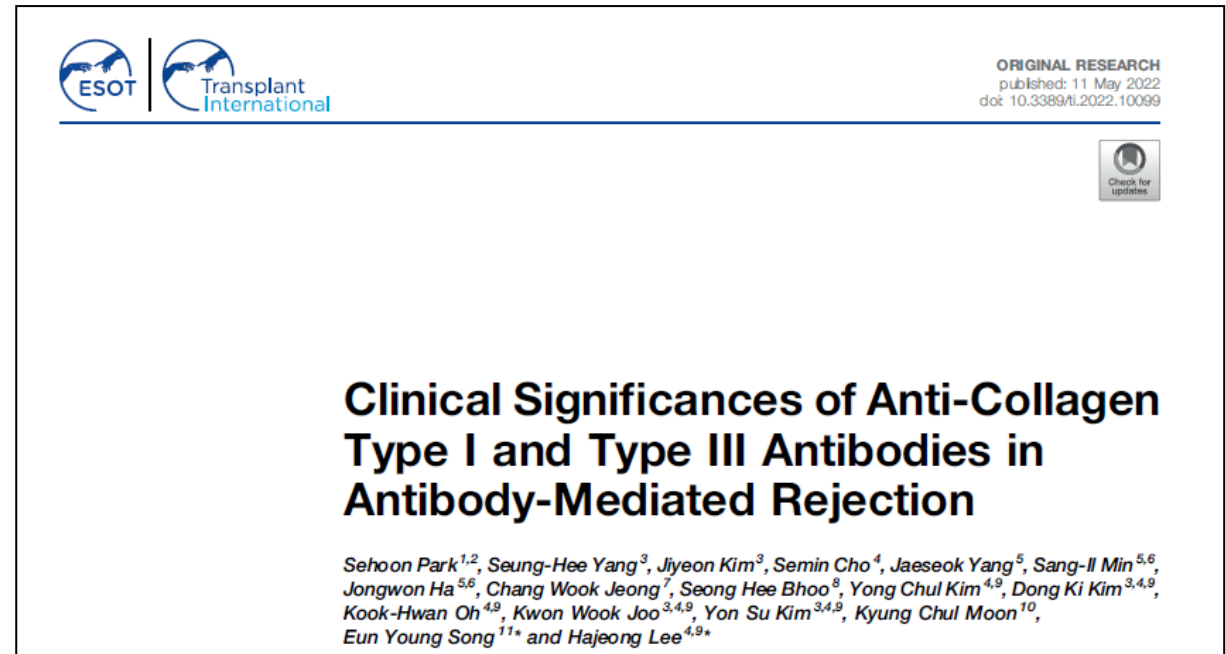


- Clinically, it is hard to determine the relevance of any positivity seen, as there is limited data.
- None of the levels would really suggest an ongoing antibody mediated rejection process
- We did note however, that the patient was positive for all of the collagen markers present in the kit.

- There is some evidence in the literature to support the role of collagen antibodies in AMR in renal transplantation
- This small study from South Korea found significant association between collagen I and III antibodies with AMR in renal transplantation, though in general the focus has been on lung transplantation.

Follow up

- At day 8 post transplant the patient had another biopsy, their creatinine was still raised at 216umol/l.
- The biopsy still showed evidence of humoral rejection but no longer had the arterial inflammation seen previously. C4d was still negative, though there were more ischaemic type changes within the glomeruli. The patient was given 5 days of plasmapheresis.
- The patient has been released back home, and continues to be monitored carefully



Case 2 – Rejection in HLA compatible tx

- Adult patient, aged 65, with renal failure secondary to hypertension and diabetes.
 - Pre-dialysis
 - Worked up for a transplant from daughter. 111 mismatch.
 - Consistently HLA antibody negative, no confirmed sensitising events.
 - Final crossmatch was negative – once autoreactivity had been accounted for by auto flow crossmatch – relevant?
-
- Transplanted 24th June 2025.

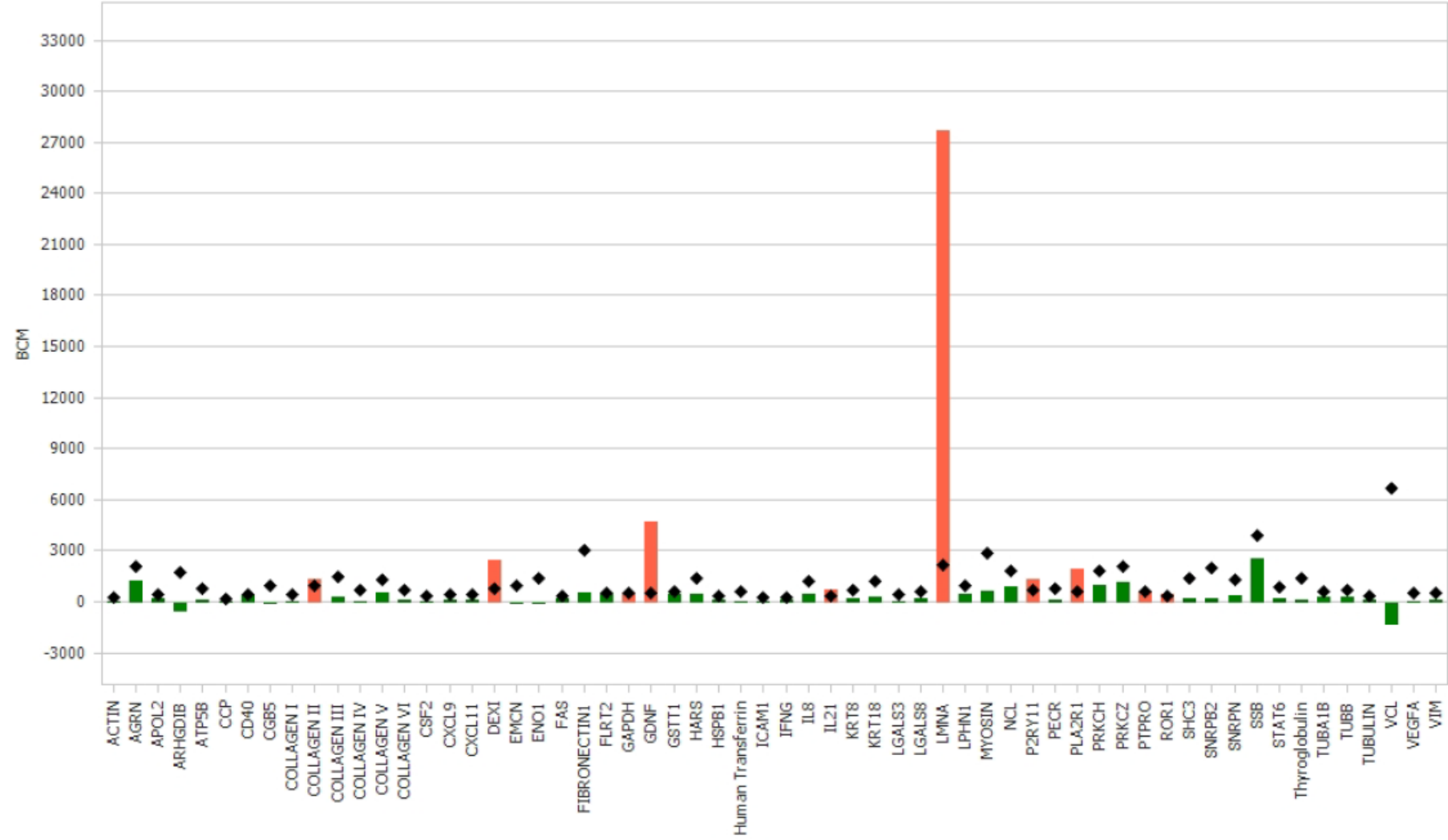
Immediate Post Tx - Rocky:

- Initially fine – UO 1000mls per hour!
- Then rapid decline on day 2 to 60ml hour.
- Ultrasound of Kidney – ok.
- Day 3 continued decline and increasing Cr. Taken back to theatre.
- Renal vein thrombus removed, open biopsy.
- Mixed Acute TCMR and 'morphology suspicious of antibody mediated processes though C4d negative.'
- Further active bleeding and return to theatre.
- Repeat ultrasound – ok.
- Further biopsy day 10 – Mixed rejection, still C4d negative.
- Pulse steroids and 10 days ATG.
- Eventually discharged 1 month post transplant – 'functioning Kidney' – Cr @420 $\mu\text{mol/ml}$
- Currently inpatient – further biopsy yesterday showing acute TCMR, no evidence of AMR.

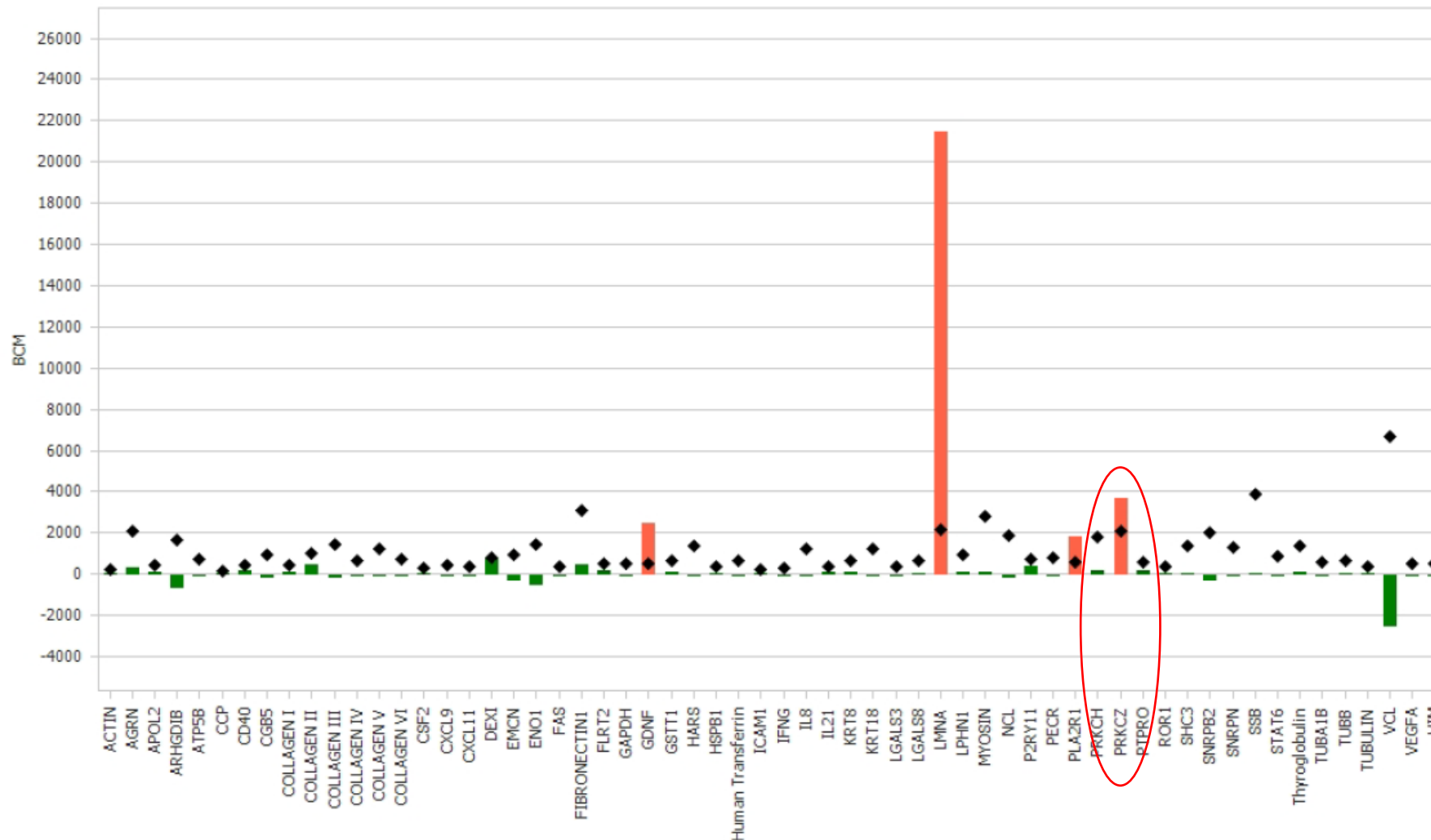
Antibodies:

- Has remained HLA antibody negative throughout.
- ‘What about non-HLA antibodies?’
- HNA negative, MICA negative – others?
- Tested immediate pre transplant, day 6 (AMR) and at 1 month (discharge) using Werfen non-HLA.

Pre Tx

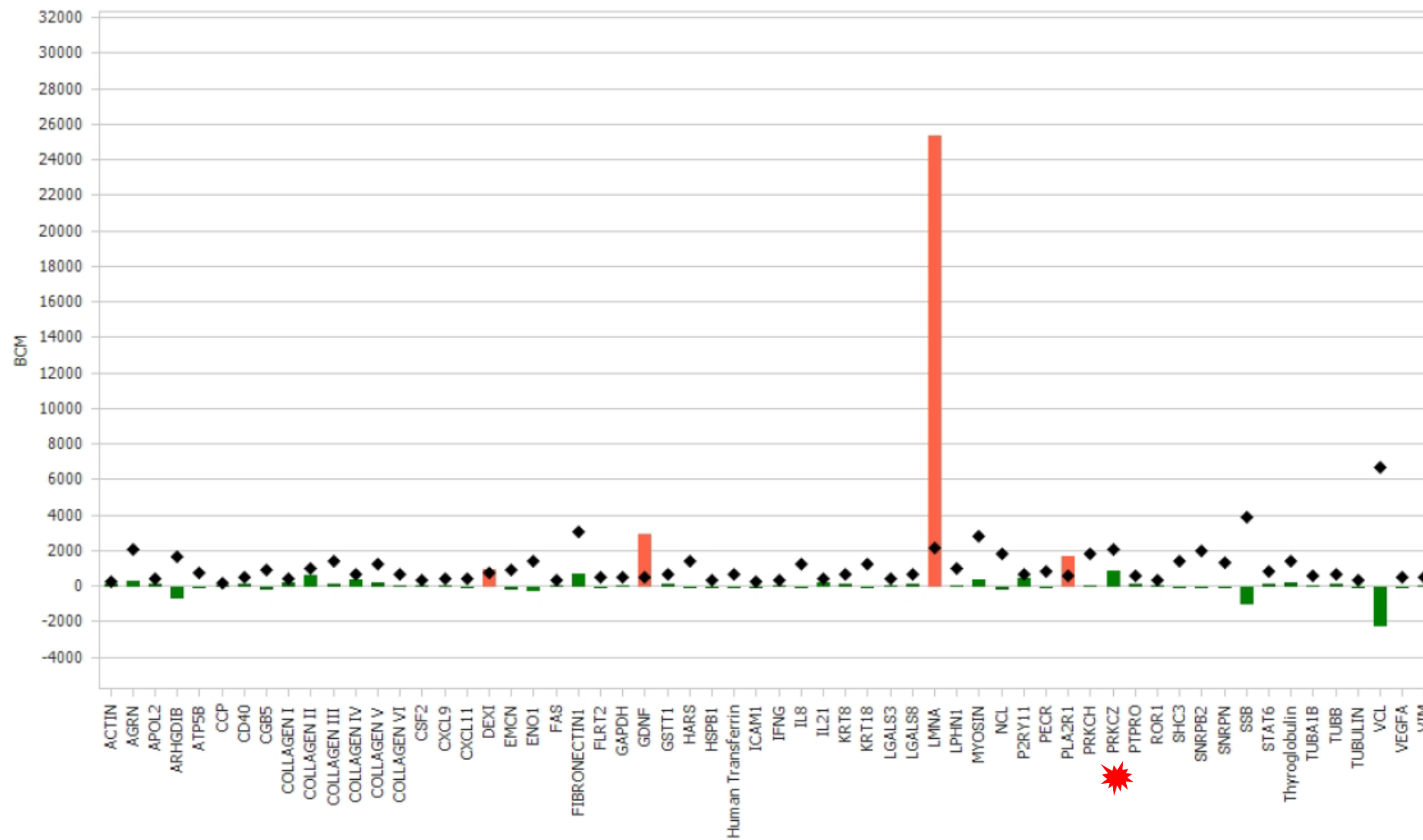


Day 6

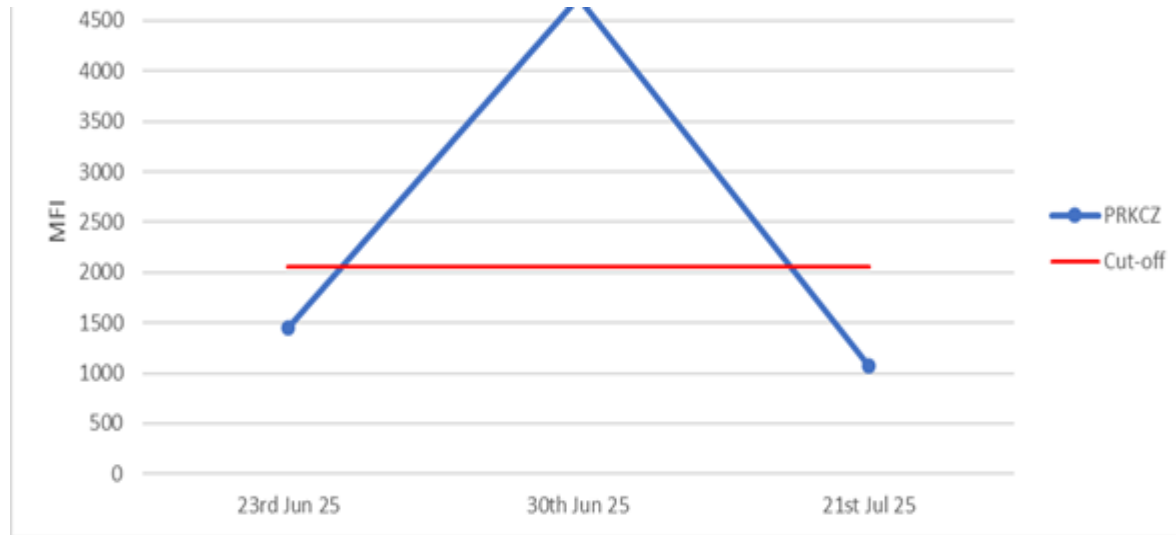


No major change –
Except PRKCZ is now positive

Discharge



PRKCZ:



Protein microarrays identify antibodies to protein kinase C ζ that are associated with a greater risk of allograft loss in pediatric renal transplant recipients

Scott M. Sutherland¹, Li Li¹, Tara K. Sigdel¹, Persis P. Wadia², David B. Miklos², Atul J. Butte^{3,4}, and Minnie M. Sarwal^{1,4}

Kidney Int. 2009 December ; 76(12): 1277–1283. doi:10.1038/ki.2009.384.

Review of literature presented cases where Ab to PRKCZ had been noted in patients at the point of acute rejection with a similar clinical picture.

15 paediatric patients with acute rejection – 3 produced high titre PRKCZ specific antibody at time of rejection.

2 of the 3 lost their grafts within 2 years compared to none of the negative group.

Not enough evidence for definitive answers – but this testing helped the clinical team with decision making.

Case 3 – Patient with previous hyperacute rejection

- A 25 year old male patient was worked up for a transplant from his mother in 2016
- 110 mismatch, no HLA DSA.
- The pair were ABO incompatible, with the patient blood group O and his mother blood group B
- The patient had very low titre blood group B antibodies (1:2), so mother considered a good candidate
- ABOi process in our centre allows standard immunosuppression protocol (Basiliximab induction, tacrolimus and MMF maintenance + steroids) in patients with titres of less than 1:8
- Flow XM was T and B cell negative

- Transplant went ahead, but did not go well
- About 20 minutes after clamps removed, the kidney suffered suspected hyperacute rejection – graft nephrectomy followed
- Investigations were performed at the time which included a post transplant XM, repeat antibody testing for HLA and antibody testing for HNA and MICA
- All lab tests were negative and no other cause for the rejection could be found
- The clinical team did not believe that the ABO antibody could be responsible for the rejection as it was at such a low level
- Fast forward to 2022 - the patient was being worked up again for listing on the deceased donor waiting list

- Between 2016 and 2022 the patient had developed some additional HLA antibody specificities, primarily against the mismatches from his first transplant
- He was activated on the deceased donor tx list in July 2022, with cRF(PRA) of 63%
- The patient spent a number of months on the list (active and suspended) before finally receiving an offer in March 2023
- Offer was from a 33yr old DBD donor, mismatch grade 011
- Crossmatch was negative for T and B cells
- In the end, the offer was declined as there were concerns still over the lack of knowledge of the cause of the 1st transplant hyperacute rejection. The clinical team and patient were nervous to move forward with another transplant
- The patient was suspended while further investigations and discussion took place

- Patient samples from -10 months, -1 week, +1 day, +4 months and current (+6 years) from date of 1st transplant were tested on the non-HLA antibody kit from Immucor.

Method Type: Standard Calculation

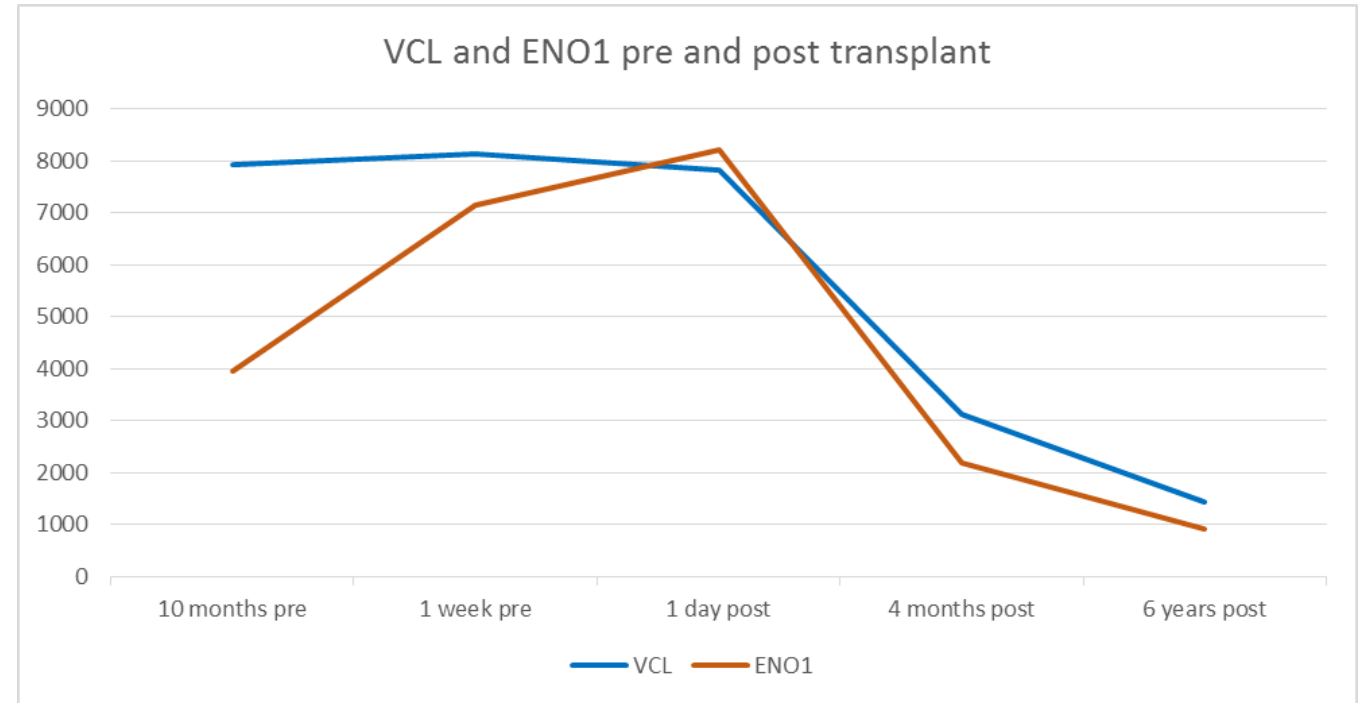
Positive CON MFI: 19384 Negative CON MFI: 212 Analysis Mode: Manual

Bead	Bead Count	Raw Value	BCM	Background	Cutoff	Assignment	Antigen
553	71	7917	6229	1688	3376	Positive	VCL
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558	156	752	326	426	852	Negative	Thyroglobulin
517	136	528	99	429	858	Negative	COLLAGEN II
525	84	496	-78	573	1146	Negative	AGRN
535	84	482	286	196	392	Negative	GSTT1
556	84	422	165	257	514	Negative	LPHN1
540	40	418	-296	714	1428	Negative	NCL
512	82	399	59	340	680	Negative	KRT18
502	73	341	-204	545	1090	Negative	SNRPN
547	78	331	-306	637	1274	Negative	HARS

- 2 of the 60 targets were positive in the pre-tx and immediate post transplant samples

- Positive targets were VCL – Vinculin, and ENO1 – Alpha-enolase
- Vinculin is a cytoskeletal protein associated with cell-cell and cell matrix junctions. It has cytoplasmic expression in most tissues, with highest levels in smooth muscle cells.
- Alpha-enolase is a glycolytic enzyme, also involved in various processes including growth control and allergic responses. It is ubiquitous and has cytoplasmic and nuclear expression
- No details found about the role of these antibodies in renal transplant, definitely need more data!
- However...

- Given the information provided by the non-HLA test, we were able to advise the clinical team that:
- Only two targets were positive in the pre, and immediately post transplant samples
- The targets are intracellular, and therefore not likely to be linked to hyperacute rejection (more likely linked to chronic processes due to accessibility).
- Levels relatively low, again not likely linked to hyperacute rejection
- Antibodies now negative



- It is most likely in this case that the hyperacute rejection was caused by ABO antibodies, despite testing showing very low titres
- There is more evidence of this currently than any other non-HLA antibody involvement
- As the following transplant for the patient would be ABO compatible there shouldn't be a repeat of the issues seen previously.
- The clinical team were reassured with the testing done in the lab, and the conclusions drawn.
- Further discussion with the patient took place, and all were happy for re-activation on the waiting list
- Patient has since been successfully transplanted.

The future

- Kits currently provide interesting insight into some difficult cases, more tests are needed to determine full usefulness.
- Clinical relevance not clear – lack of evidence.
- What do these antibodies look like in healthy or non-rejecting transplant patient population?
- What cut-offs are relevant in the clinical setting?
- Is it the presence of antibody, or changes to antibody level that are relevant (if at all)
- Are there gender variations?
- Are there ethnic variations?
- Lots of other potential targets – definitely not an exhaustive list
- Need more data!

Published Data

- To date there are an increasing number of publications focusing on association with rejection.
- Largely these are limited by population size and biased patient selection.
- We know of two larger scale studies (with both manufactures kits) that have indicated that pre transplant non-HLA antibody burden could be linked with outcome and rejection episodes.
- Likely impact of HLA and non-HLA working together.
- 14th International workshop data hinted at possible associations with persistent non-HLA antibody associating with rejection episodes.
- All call for establishment of wider larger scale studies.

What's missing?

- We feel that the clinical utility of the kits isn't going to be realised until we get a better understanding of what 'normal' looks like.
- Suggestion that non-HLA targets linked with ESRD (Higher), HD vs PD (higher), previous transplant (lower).
- Likely differences due to cause of ESRD – immune vs non-immune.
- Potential ethnic diversity – largely not investigated in current studies.
- Potential gender diversity – largely not investigated in current studies.
- We would like to add to the knowledge.

The plan - OuTSMART

- At Guys we were centrally involved in the recently published OuTSMART Study.

Optimized immunosuppression to prevent graft failure in renal transplant recipients with HLA antibodies (OuTSMART): a randomised controlled trial



Dominic Stringer,^{a,b} Leanne Gardner,^{b,c} Olivia Shaw,^d Brendan Clarke,^e David Briggs,^f Judith Worthington,^g Matthew Buckland,^h Guilherme Danzi,ⁱ Rachel Hilton,^j Michael Picton,^k Raj Thuraisingham,^l Richard Borrows,^m Richard Baker,ⁿ Keith McCullough,^o John Stoves,^p Mysore Phanish,^q Sapna Shah,^r Kin Yee Shiu,^s Stephen B. Walsh,^s Aimun Ahmed,^t Waqar Ayub,^u Janet Hegarty,^v Rose Tinch-Taylor,^{a,b} Evangelos Georgiou,^b Natalie Bidad,^w Ayşenur Kılıç,^w Zoe Moon,^w Robert Home,^w Paul McCrone,^{b,x} Joanna Kelly,^b Caroline Murphy,^b Janet Peacock,^{y,z} and Anthony Dorling^{c,*}



OuTSMART

- In essence this was a large study to investigate the utility of optimisation of immunosuppression to improve long term outcomes in patients who became HLA DSA positive.
- Open label, biomarker led, double blind randomised trial in 13 UK centres.
- Eligible patients included adults who were at least 12 months post transplant who had not been treated for AMR in past 6 months.
- 2037 patients were enrolled and randomised based on HLA antibody status at enrolment, current immunosuppression and location.
- Blinded patients received standard of care.
- Unblinded patients who were DSA positive at enrolment were educated on adherence, had their IS optimised and were given a steroid boost.
- Screening continued every 8 months for HLA antibody negative patients – those who became positive underwent IS optimisation.
- All patients had a minimum follow up of 32 months – restarted for those who became pos.

OuTSMART

- Overall 15-20% of the patients with DSA lost their grafts within the study period compared to 7% of the HLA antibody negative patients.
- The results confirmed that the development of HLA DSA was a prognostic marker of future graft failure.
- Optimisation of IS following detection of DSA did not improve outcomes in the unblinded arm compared to standard of care.
- However, BPAR was reduced in the DSA positive patients in the unblinded arm.
- This has left us with a biobank of samples from a very well defined renal transplant population with research consent.
- Work underway includes further dissection of those who were DSA positive including antibody characteristics and immunological cell activity.

Non-HLA

- We have begun to investigate the non-HLA antibody characteristics of the population.
- Samples from pre transplant, study enrolment and study exit will be tested using the non-HLA antibody kit from Werfen.
- Additional samples will be collected from healthy volunteers – living donors – to create a ‘normal healthy population’ control group (n=1-200)
- Patient demographics, HLA antibody results, Immunosuppression treatment and biopsies (where done) are readily available.
- From this testing we aim to –
 - Identify the baseline characteristics of the antibodies in this diverse transplant population.
 - Identify the baseline characteristics of a healthy population.
 - Add to the knowledge of commonly seen antibodies, associations with different patient demographics and disease states.
 - Further analyse associations of specific antibodies with longer term outcomes and the timing of the antibody production with pre and post transplant status.
 - Locally the aim will be to try to establish the patient populations in whom testing is useful and to try and be able to answer (for ourselves at least) – what the results actually mean and to be able to answer the question ‘What about the non-HLA?’ with a bit more certainty.

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