The reduction in immunosuppression laboratory dilemma

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Introduction

Since the implementation of virtual crossmatch (vXM) in Australia (February 2023), new challenges have presented themselves to the tissue typing laboratories. The absence of a physical crossmatch at the time of deceased donor matching has put increased emphasis on the Luminex single antigen bead assay results. To facilitate vXM in Australia, transplant wait list (TWL) recipients are screened by Luminex Single Antigen Beads at 90 day intervals. These fixed timepoints create uncertainty in recipients undergoing immunosuppression withdrawal where the natural history of antibody changes is not well documented.

Case Presentation

60yo female previously transplanted renal recipient (6/6 mismatch), returns for a second graft presenting with no Class I human leukocyte antigen (HLA) antibodies and strong DQB1 Class II HLA antibodies. Calculated panel reactive antibody (cPRA) at time of relisting assessment was 63%. However when recipient was ready to be activated 8 months later the cPRA had increased to 83%. After consulting with the clinical unit, it was discovered that the recipient's immunosuppression had been ceased at day 301 post initial screening. Ongoing quarterly screening was performed while the recipient was active on the TWL, repeated increases in the cPRA were observed, reaching 99% over time. The new specificities appeared to be attributed to the HLA-A, B, DRB1 and DRB3 mismatches.

HLA typing recipient		HLA typing 1st graft	
A*11:01	A*03:01	**A*01:01**	**A*02:01**
B*51:01	B*07:02	**B*08:01**	**B*44:03**
C*15:02	C*07:02	C*07:01	**C*03:04**
DRB1*04:04	DRB1*15:01	**DRB1*03:01**	**DRB1*13:01**
DQB1*03:02	DQB1*06:02	**DQB1*02:01**	DQB1*06:03
DQA1*03:01	DQA1*01:02	**DQA1*05:01**	DQA1*01:03
DPB1*03:01	DPB1*16:01	**DPB1*02:01**	**DPB1*04:01**
DPA1*01:03		DPA1*01:03	
DRB4*01:03	DRB5*01:01	**DRB3*01:01**	**DRB3*02:02**

Figure 1. HLA typing for recipient and 1st graft; mismatches highlighted in bold

In discussion with the clinical unit, due to the recipient's fragility and unstable antibody profile, a conservative approach was agreed upon to restrict access to donors where antibody development was anticipated due to recent donor specific antibody (DSA) positive offers. The standard unacceptable antigen (UA) threshold used in Australian centres is 4000 mean fluorescence intensity (MFI), however in this case a lower UA threshold in conjunction with shared eplet reactivity was applied.

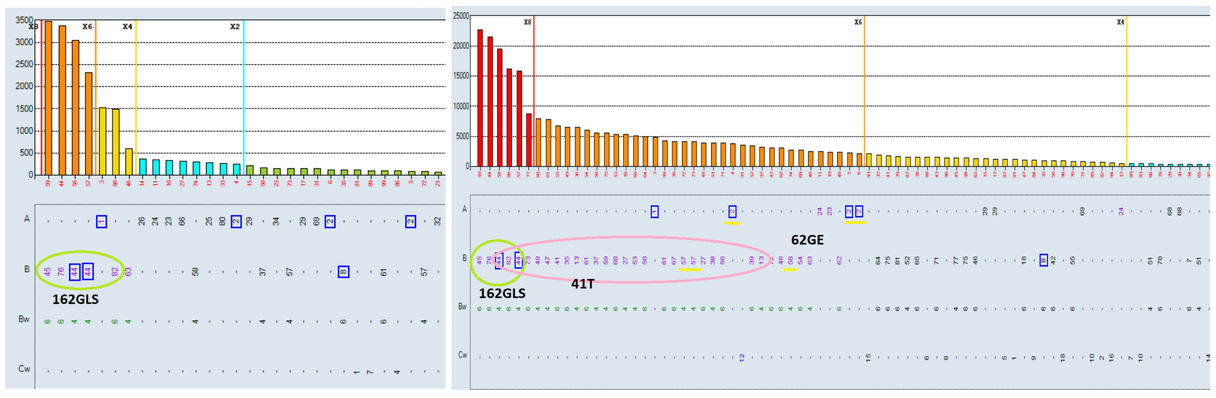

Figure 2. Comparison of Class I antibody profile at day 379 and 713 post reenrolment. Previous transplant mismatches circled in blue.

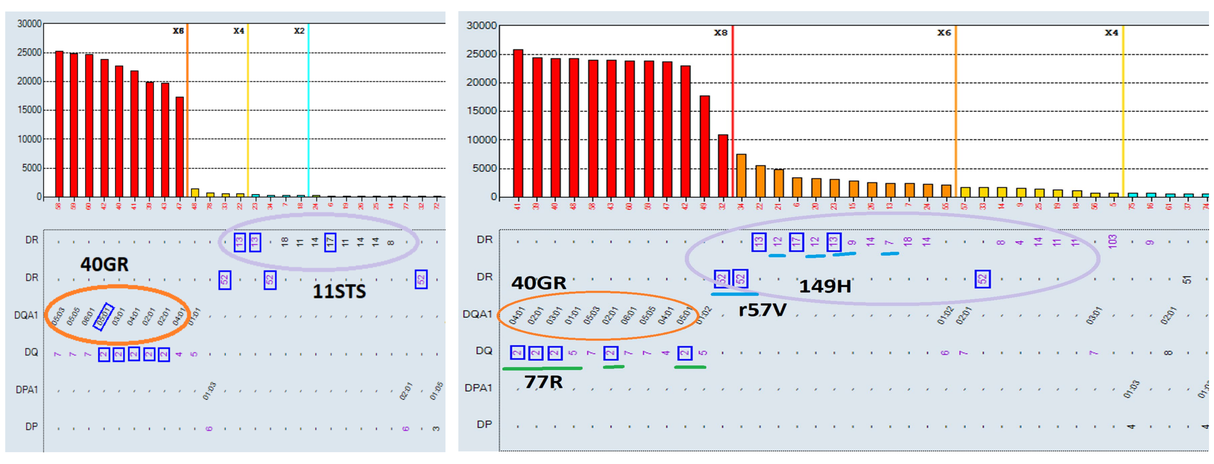

Figure 3. Comparison of Class II antibody profile at day 379 and 713 post reenrolment. Previous transplant mismatches circled in blue.

Day	Event	cPRA	UA criteria	DSA at time of DD offer	DSA at next screening time point
0	Reenrolment - Initial screening	63%	Standard >4000 MFI UA	-	-
247	Restart dialysis	-	-	-	-
301	Ceased immunosuppression	-	-	-	-
379	Confirm Ab profile prior to relisting	83%	>4000 MFI UA	-	-
419	Activated on TWL - Listing screening	89%	Standard >4000 MFI UA	-	-
474	DD match (Accepted by higher ranks)	-	-	1x DSA (historical DSA only, no current DSA)	New DSA at day 496 = 2646 MFI
496	Quarterly screen	96%	Standard >4000 MFI UA	-	-
587	Quarterly screen	96%	Standard >4000 MFI UA	-	-
633	DD match (Declined)	-	-	2 x DSA (734 & 2826 MFI as of day 587) - Declined	DSA level at day 650 = 1516 & 5181 MFI
649	DD match (Declined)	-	-	2 x DSA (965 & 3217 MFI as of day 587) - Declined	DSA level at day 650 = 1990 & 5186 MFI
650	Bimonthly screen & clinical discussion	97%	Lower threshold - CI Eplet UA profile	-	-
664	Further clinical discussion	99%	Lower threshold - CI & CII Eplet UA profile	-	-
713	Bimonthly screen	99.4%	Lower threshold - CI & CII Eplet UA profile	-	-
718	DD not matched	-	-	1 x DSA listed as UA (MFI = 3488)	DSA level at day 741 = 4897 MFI
718	DD not matched	-	-	1 x DSA listed as UA (MFI = 2428)	DSA level at day 741 = 1528 MFI
741	Monthly screen, placed On Hold (Clinical Reasons)	99.2%	Lower threshold - CI & CII Eplet UA profile adjusted C* removed to allow offers)	-	-
832	Quarterly screen, Nearing reactivation	99.8%	CI & CII Eplet UA profile	-	-

Figure 2. Timeline of events from reenrolment, displaying corresponding cPRA with each sample screened, UA criteria applied, and retrospective review of potential deceased donor (DD) offers comparing DSA at time of offer to the DSA at the next screening time point.

Definitions: cPRA, calculated panel reactive antibody; DSA, donor specific antibody; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; TWL, transplant wait list; UA, unacceptable antigen; vXM, virtual crossmatch

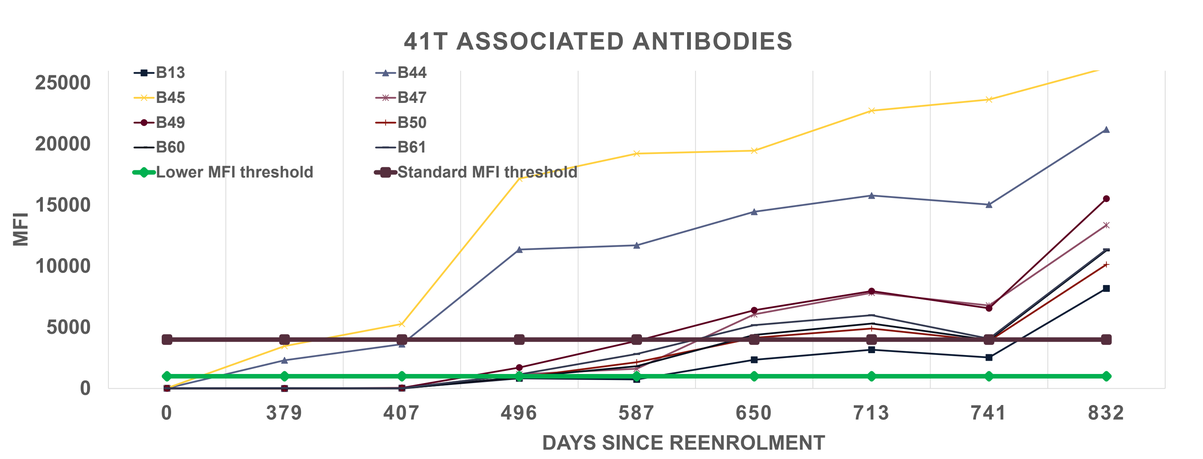

Figure 4. tracking of 41T associated antibody development over time

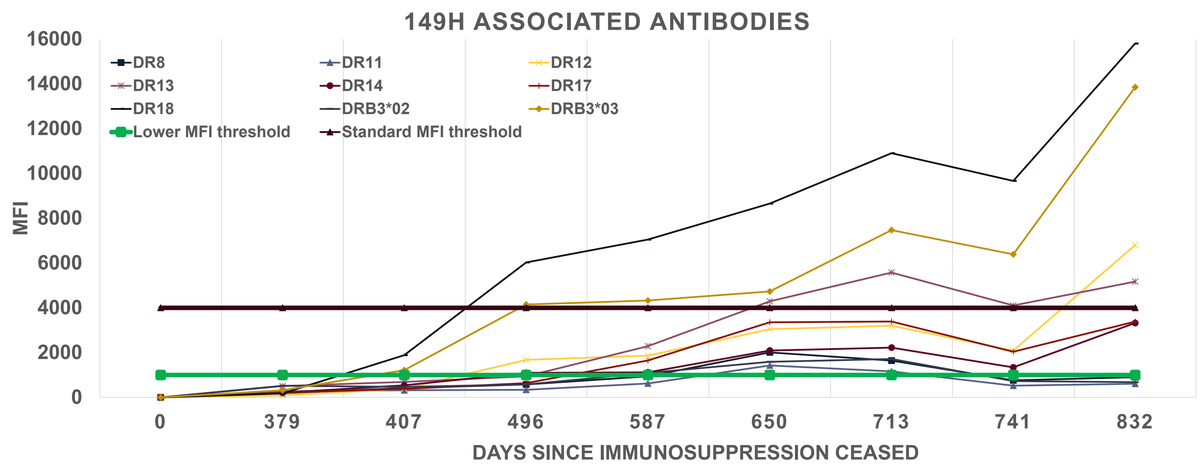

Figure 5. tracking of 149H associated antibody development over time

Further Investigation

While previous transplant mismatches are a known sensitisation source, anticipating the associated antibodies that will develop is difficult. Epitope analysis was used to identify specificities below the standard UA threshold which were associated to previous transplant mismatches. At day 379, the Class I profile appeared to be contained to a 162GLS eplet relating to the B*44 mismatch. However by day 713, additional specificities also attributed to the B*44 were now detectable (41T) with A*02 mismatch associated 62GE developing. The recipient had an established HLA-DQ profile with weaker HLA-DRB1 specificities associated to mismatch DRB1*13, DRB1*03:01 and DRB3 (11STS). However at day 713, what initially was thought to be 11STS profile developing, ended up being eplet reactivity to 149H, a much broader DRB1/DRB3 eplet with 77R and r57V to explain additional DQB1*05 and DRB1*07/09/DRB3 specificities respectively. The DQB1 specificities were observed to change more rapidly compared to the HLA-A, B, and DRB1 where the changes were more gradual. Surrogate flow crossmatches (Halifaster) demonstrated potential DSA positive offers between 1000 to 4000 MFI were associated with weak to moderate positive channel shifts from threshold (data not shown). In the context of the MFI changing over time, the 90 day gap in screening does create uncertainty for the accepting clinical unit if approaching the next screening timepoint, therefore the lower UA threshold does add another layer of protection.

Observations and Conclusions

There is minimal detailed information on how HLA laboratories manage TWL patients undergoing immunosuppression withdrawal. While excluding previous transplant mismatches mitigates the risk to an extent, DSA associated to these mismatches through shared eplets may be underestimated. Complete immunosuppression withdrawal clearly has a close association with sensitization increase in cPRA[1], where the probability of receiving a transplant is inversely related to the % PRA[2]. While finding the balance between increased risk of allo-sensitisation and complications associated with ongoing chronic immunosuppression[3] remains difficult, further studies are required to understand the relationship between immunosuppression withdrawal and changes and its effect on HLA antibody development. In our centre, retransplant renal recipient routinely undergo immunosuppression weaning or cessation while active on the TWL. This case study demonstrates that eplet analysis can be used to predict antibody development and provide a safety net while antibody profile is in flux. While increased antibody surveillance is recommended during immunosuppression weaning, the logistics of this remain challenging and is not always feasible[4], applying a conservative approach to listing unacceptable antigens could be a viable alternative option. Management of these recipients is complex and time consuming and requires close collaboration between the clinical unit and HLA laboratory.

References

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Acknowledgement

Royal Melbourne Hospital Renal Unit
Scientists at Victorian Transplant and Immunogenetics Service, Australian Red Cross Lifeblood

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Australian governments fund Australian Red Cross Lifeblood to provide blood, blood products and services to the Australian community.