UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

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Interpretive Educational Scheme (iED)
Clinical Scenario 1/2020 – Cardio-thoracic Transplantation

Dispatched on 1st September 2020

Summary of Results

A total of 45 responses were received, 20 from UK & Ireland (UK&I) based laboratories and 25 from Rest of the World (RoW).

A potential cardio-thoracic donor is offered to your centre on 07/01/2020.
The donor is Female, 64 years old and ABO blood group O.
The donor HLA type is: HLA-A2, A11; -B27; -Cw1; -DR15, DR103; DR51; -DQ6, DQ7; -DPB1*03:01, DPB1*10:01

Question 1

The Transplant Co-ordinator asks you to assess the following recipients. All patients have the same clinical urgency:

Recipient	ABO	Organ Req'd	Antibody Positive	Donor Directed (Peak MFI)	Date of Last Sample
A	A	Heart	Yes	Yes (DR15 - 12500)	26/11/2019
B	O	Heart	Yes	No	03/01/2020
C	O	Double Lung	Yes	Yes (Cw1 - 1989)	27/11/2019
D	A	Heart	No	No	14/10/2019
E	O	Single Lung	Yes	Yes (B27 – 13716, A2 – 3095, A11 - 1662)	26/11/2019
F	O	Heart	Yes	Yes (DQ6 - 7500)	03/01/2020
G	A	Heart	Yes	Yes (DP3 - 2150)	31/10/2019

1.1 Rank the three most suitable recipients based on the information provided and give reasons for the choices made:

Rank	Recipient	Total (%)	UK&I (%)	RoW (%)	Most Common Reasons
1st	**B**	**41 (93%)**	20 (100%)	21 (84%)	Recent sample. Patient sensitised, may not get another suitable offer. No DSA. ABO compatible. Standard risk. Check if any sensitising events since last sample.
	D	**4 (7%)**	0	4 (16%)	No antibodies. ABO compatible.

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2nd	**D**	**28 (62%)**	18 (90%)	10 (40%)	No DSA. ABO compatible. Tested within 3 months. Patient not sensitised, likely to receive other offers. Confirm no sensitising events since last antibody screen. Standard Risk.
	C	**13 (29%)**	2 (10%)	11 (44%)	ABO matched. Low level DSA against HLA-C. Recent antibody screen.
	B	**4 (9%)**	0	4 (16%)	No DSA. ABO compatible.
3rd	**G**	**15 (33%)**	10 (50%)	5 (20%)	ABO compatible. Favourable CTAG* Level (Level 3). Recipient C has higher cumulative MFI when Cw1 homozygous MFI doubled. Lower DP expression potentially more permissible. DP expression by cardiac cells is lower than a number of other cell types cell. Low risk of hyperacute rejection.
	C	**17 (39%)**	6 (30%)	11 (44%)	ABO match. Total MFI<2000. Donor is Cw1 homozygous so DSA MFI of 3978, risk level III in BTS/BSHI guidelines. Minimum risk of hyperacute rejection. Low level donor HLA specific antibodies.
	D	**6 (13%)**	2 (10%)	4 (16%)	ABO compatible. HLA antibody negative.
	E	**1 (2%)**	1 (5%)	0	Antibody test performed within 3 months. HLA-Cw1 donor specific antibody has an MFI value less than 2000. The risk level is I: Standard.
	F	**4 (9%)**	0	4 (16%)	ABO compatible. DSA against DQ. Recent antibody screening.
	None	**2 (4%)**	1 (5%)	1 (4%)	Could not select a 3rd recipient without discussion with the clinical team.

***CTAG = Cardiothoracic Advisory Group Guidelines, NHS Blood and Transplant**

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1.2 Explain why the other recipients were not selected.

Recipient	Comments
A	• Antibody directed to DR15 (MFI 12,500) • CTAG Level 4: veto to transplant except for exceptional cases. • Increased chance of Hyperacute AMR. • CII Ab can be treated also evidence of accommodation. • Different blood group. • Likely positive B cell CDCXM.
B	• No comments received.
C	• Antibody directed to Cw1 (cumulative MFI 3,978; Cw1 homozygous so MFI doubled). • CTAG Level 3: low risk of hyperacute rejection but significant risk of early rejection/AMR. • Low risk of hyperacute rejection • More recent sample would need to be obtained for screening. • Lung recipient and low level Cw DSA. • Low risk lung transplant offers would not be considered by clinicians at this centre.
D	• Recipient will have plenty other opportunities for transplantations (blood type A with no antibodies, so with cPRA=0%). • Different blood group.
E	• Multiple donor-specific antibodies (cumulative MFI 32,189; B27 homozygous so MFI doubled) 'High' level B27 donor homozygous, 'Medium' level A2 and 'Low' level A11. • CTAG Level 4: veto to transplant except for exceptional cases. • Increased chance of Hyperacute AMR. • Likely cause a positive B- and T-cell CDC-crossmatch.
F	• Antibody directed to DQ6 (MFI 7,500). • CTAG Level 4: veto to transplant except for exceptional case. • Increased chance of Hyperacute AMR. • CII Ab can be treated also evidence of accommodation.
G	• Preformed Class II DSA gives an increased risk of cardiac allograft vasculopathy. • Donor specific anti HLA-DP3 antibody, MFI 2150, detected within 3 months. • CTAG Risk Level III. • ABO compatible (blood group A with access to group O and A donors). • Significant risk of early rejection and antibody mediated graft damage. • Could be considered if immediate antibody reduction feasible. • Cw and DPB1 are low expression antigens, BUT would require discussion with the clinical team.

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Question 2

The heart was accepted for a super urgent patient at another centre. The antibody results for the remaining two lung patients were provided.

2.1 For the potential recipients please select the immunological risk for each recipient and explain the reason for each selection:

Recipient	Risk	Total	UK&I	RoW	Reason
C	CTAG* Level 3	12 (27%)	12 (60%)	0	Cumulative MFI 3,978. Cw1 homozygous so MFI doubled. Lower expression HLA-Cw potentially more permissible. Low risk of hyperacute rejection but significant risk of early rejection/AMR.
	CTAG Level 2	3 (7%)	2 (10%)	1 (4%)	Cw1 antibody at 1989 MFI. Minimum risk of HAR due to low level DSA. If following BSHI/BTS guidelines in doubling MFI due to homozygosity then would be risk level III.
	Medium / Intermediate Risk	4 (9%)	1 (5%)	3 (12%)	Weak DSA Cw1 MFI 1989. FCXM likely to be negative. Check any sensitisation since the last sample.
	Low / Standard Risk	25 (55%)	4 (20%)	21 (84%)	Cw1 = 1989. Cumulative MFI (doubling Cw1) just over 4000. Minimum risk of hyperacute rejection due to low level DSA but greater than standard risk of rejection.
	No response	1 (2%)	1 (5%)	0	N/A
E	CTAG Level 4	16 (36%)	16 (80%)	0	The patient has a B27 DSA of 13,000 MFI, combined with an A2-3000 MFI and A11-1662 MFI. Cumulative donor-directed MFI is 32,189. Risk of hyperacute rejection, contraindication to transplantation. Tx veto, apart from exceptional cases where risk should be further refined e.g. complement fixing antibodies. Crossmatch likely positive. The patient has a reactive public epitope against Bw4.
	High Risk	24 (54%)	2 (10%)	22 (88%)	Multiple DSA with peak DSA at >13,000 MFI. Cumulative MFI almost 20,000. High risk of hyperacute rejection.

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	CTAG Level 3	1 (2%)	0	1 (4%)	HLA alloimmunisation positive, CDC ab: unknown, PRA >50%, Unacceptable ag: Positive, risk HLA: positive , Virtual XM : positive.
	Medium/ Intermediate Risk	3 (6%)	1 (5%)	2 (8%)	High level DSAs: B27 MFI 13716, A2 MFI 3095, A11 MFI 1662 (cumulative ~20,000). CDC/FCXM likely to be positive.
	No response	1 (2%)	1 (5%)	0	N/A

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Patient C was selected for a transplant.

2.2 Would you perform a prospective crossmatch for this patient based on the HLA antibody results and give reasons for your answer:

Answer	Total	UK&I	RoW	Most Common Reasons
Yes	**20 (44%)**	6 (30%)	14 (56%)	*UK&I:* • Presence of DSA - Cw1 DSA present in most recent sample, and not tested for 5 weeks. • Antibodies are well defined but close to 5000 MFI. Crossmatch is advisable if time allows. • Prospective VIRTUAL crossmatch - all sensitised patients receive a virtual crossmatch to assess for any DSA and stratify risk as per CTAG guidelines. • If local donor or where donor blood can be sent in advance of retrieval perform prospective flow cytometry crossmatch. • Confirm clinical significance of single antigen bead results in crossmatch (Cw1 bead sometimes false positive in LabScreen tests). *RoW:* • Presence of DSA. • Determine if the DSA can bind and activates complement. • To see the strength of the DSA. • The presence of a positive virtual crossmatch and the MFI values indicate a high sensitization for DSA. • In the sample a DSA was identified that despite the low MFI value might be prone to a Hook effect. • In case of recent immunizing events and/or absence of HLA antibody history, we perform a prospective physical crossmatch.

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				• The crossmatch results can guide desensitisation protocols of patient and which immunosuppressive regime to employ. • We would advise a prospective cytotoxic crossmatch. If the Cw1 is not active in a CDC assay, the prospective crossmatch could be omitted.
No	**25 (56%)**	14 (70%)	11 (44%)	*UK&I:* • Local policy is to not prospectively crossmatch cardiothoracic transplants except for exceptional cases. • Crossmatch is limited by logistics in obtaining donor material from imported donors. • The transplant is low risk and cold-ischaemia time must be minimised. • Predict a negative crossmatch. • A virtual crossmatch could be performed as long as there had been no sensitising events since the last antibody. • If sensitising events since last sample; current patient serum could be tested by Single Antigen antibody definition kits prior to issuing a virtual crossmatch. • The crossmatch result would not change the decision to transplant, it could therefore be performed retrospectively. • The crossmatch would aid as a guide to post-transplant management. • Crossmatch is likely to be positive due to the presence of donor directed antibody. Our clinical team would make the decision on whether to proceed, based on the risk level provided by us using the cumulative MFI values for the donor directed antibody. • MFI of this level of Cw would give a negative result in Flow cytometric crossmatch. • Patient is relatively non-sensitised; Peak defined specificity Cw12 MFI 3511 (pk bead MFI 12511 UD) CDC/FCXM likely to be negative due to weak DSA in the last sample 27.11.19). • Request a current sample for SAB analysis to confirm MFI hasn't changed since last screened. *RoW:* • Perform prospective crossmatch depending on whether complement fixing antibodies are identified in the CDC screening and/or an immunological event such as blood transfusion occurred after the date of last tested sample (27-11-2019).

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				• The patient is only mildly immunized with few week antibodies (< 3000 MFI), which will not give rise to a positive CDC-crossmatch. We would therefore only perfom a virtual crossmatch, which would be considered negative with DSA MFI < 3000. • As the immunological is low, we favour the shortest cold ischemia time. • Cold ischaemia delay must be less than 8 hour for thoracic grafts. • DSA low MFI and would probably not be detected by CDC Crossmatch.

Question 3

The crossmatching results for Patient C were provided.

3.1 Would these results change the risk level assigned in Question 2?

Response	Total (%)	UK&I (%)	RoW (%)	Selected Comments
Yes	**8 (18%)**	3 (15%)	5 (20%)	• Historic positive, current equivocal flow cytometric crossmatch in the presence of donor specific antibodies. • Autologous crossmatch results could affect the interpretation of the results. • Intermediate risk. • Information about viral infection or medication would be useful to assess the risk.
No	**35 (78%)**	16 (80%)	19 (76%)	• The patient has an IgM antibody, this is not a veto to transplantation. • Further information on the patient's sensitisation history required. • Auto-crossmatch required. • Reactivity pattern of the Flow Crossmatch results is not consistent with MFI of the peak donor directed single antigen bead result. • In the absence of an auto Flow Cytometric crossmatch results suggest IgM and possibly non-HLA or auto-reactive antibodies being present - none are defined. • We would advise about and discuss the IgM rise in current sample as detected by CDC and a rise of B cell

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				IgG by FCXM which may have gone undetected by the less sensitive CDC. • Class II Antibody class switching to IgG may be in progress, as MFI of Cw1 and T cell FCXM strength have remained unchanged. • Class II antibodies are well documented to be associated with negative outcome in lung transplantation and BOS.
Unclear	**2 (4%)**	1 (5%)	1 (4%)	

3.2 What would you suggest, if anything, to the clinical team to increase the chances of a successful transplant?

Recommendations	Total (%)	UK&I (%)	Row (%)
Augmented Immunosuppression	**22 (30%)**	16 (37%)	6 (19%)
Desensitisation Therapy Pre-transplant	**8 (11%)**	4 (11%)	4 (13%)
Post-Transplant DSA Monitoring	**31 (42%)**	18 (42%)	13 (42%)
Test for IgM HLA Antibodies	**2 (3%)**	2 (4%)	0
Perform C1q Assay	**2 (3%)**	1 (2%)	1 (3%)
Test for non-HLA Antibodies	**1 (1%)**	1 (2%)	0
Perform an Autologous Crossmatch	**4 (5%)**	1 (2%)	3 (10%)
Test Graft Function More Frequently	**1 (1%)**	0	1 (3%)
No Comments	**3 (4%)**	0	3 (10%)

Question 4

Single antigen bead testing was performed on post-transplant sample received. The results are provided.

4 Based on these results what would your recommendations be for further immunological monitoring?

Recommendations	Total (%)	UK&I (%)	Row (%)
Long Term Post-Transplant Monitoring	**41 (73%)**	20 (71%)	21 (75%)
Retest Samples (and check for Prozone)	**6 (11.5%)**	3 (11.5%)	3 (11%)
Investigate Immunosuppression Level has Affected MFI Levels	**2 (4%)**	1 (3.5%)	1 (3.5%)
Monitor Lung Function	**2 (4%)**	1 (3.5%)	1 (3.5%)
Investigate Whether Virus has Affected MFI Levels	**1 (1.5%)**	1 (3.5%)	0
Check Measurement of Uncertainty	**1 (1.5%)**	1 (3.5%)	0
Test for non-HLA	**1 (1.5%)**	1 (3.5%)	0
Re-perform Crossmatching	**1 (1.5%)**	0	1 (3.5%)
Adapt Immunosuppression	**1 (1.5%)**	0	1 (3.5%)

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Question 5

5 Does your Laboratory perform Cardiothoracic transplantation?

Response	Total (%)	UK&I (%)	RoW (%)
Yes	**27 (60%)**	6 (30%)	21 (84%)
No	**18 (40%)**	14 (70%)	4 (16%)

General Comments

- Would also enquire about recent sensitisation events.
- All patients in question 1 would have had HLA antibody testing prior to the offer as all samples were >6 months old. Assumed they were in date and no sensitising events since last tested for the purpose of the scenario. This question would have been asked of the transplant coordinator.
- As we are not a Cardiothoracic centre it was interesting to see this scenario.
- Information on sensitising events and gender of patients may have been useful. Patient C bead 48 (B27, donor relevant) data missing on page 2.
- We only perform cardiac transplants so the inclusion of lung recipients was a useful exercise.
- Our centre uses different MFI levels to stratify HLA antibodies than the CTAG guidelines as locally agreed with the transplant team; 'Neg' = <1000 'Low' = 1000 - 1999 'Medium' = 2000 - 3999 'High' = > 4000 A virtual crossmatch will be issued for sensitised cardiothoracic patients where up to 2 x 'Low' OR 1 x 'Medium' MFI level specificities are detected in the last sample. We would only perform a prospective crossmatch where patient HLA specificities couldn't be clearly defined.
- In most cases, patient's clinical urgency is taken into consideration along with immunological risks when choosing a patient for a given offer.
- Useful to know genders of potential recipients, especially for heart Tx; useful to know donor Bw4/Bw6, especially in relation to B27 for interpretation of Ab data.
- We only perform heart transplantation and hence only perform the investigations and follow-up for HTX-patients and not for lung transplantations.
- We perform only heart transplantation in our centre and we are not used to have clinical discussion on lung transplantation.
- Our clinical team was involved in answering the questions.
- Please note, our responses are primarily based on Australian methods for assessing transplantation criteria. These are also Tx centre dependent. Thank you for allowing us to participate!
- We do not perform lung transplant, only heart.

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Comments and suggested results from UK NEQAS for H&I

Question 1

Order	Recipient	Reason
1	B	Recipient B has no donor directed antibodies and last sample was 4 days ago (CTAG standard risk)
2	D	Recipient D has no donor directed antibodies but ranked below B as the last serum sample was 14/10/2019 (CTAG standard risk)
3	G	The remaining recipients all have donor directed antibodies. Recipient G has been ranked 3rd as it has the lowest MFI

Comment:
3rd choice could also be Recipient C as there is minimal difference in the MFI between C and G and Recipient C's last sample was nearer to the offer date.

Recipient A, E and F were not selected as they all have donor directed antibodies which, based on the MFI data, would represent too great an immunological risk (CTAG risk level IV).

Question 2

Recipient	Risk	Reason
C	Intermediate Risk (CTAG Level III)	Donor directed antibody against Cw1 with a peak MFI of 1989. As the donor is homozygous this is doubled to give cMFI of 3978.
E	High Risk (CTAG level IV)	The patient has antibodies directed against donor mismatches that result in a cMFI for this patient of 32,186.

This patient would not require a prospective crossmatch - the last sample tested was within the last 6 weeks, the clinical team confirmed no sensitisation events since that date. Prospective crossmatching would delay the transplant.

Question 3

The crossmatch results for sample 22/01/2018 are the area for concern – the cytotoxic crossmatch reduced with DTT but was not completely abrogated and the flow crossmatch is B cell positive. However, the MFI data is not supportive of a CDC crossmatch positive. The day of transplant sample is negative for IgG donor directed antibodies in the CDC assay and in the flow crossmatch raised but did not reached test cut off. Overall this would not change the risk level.

Suggestions to the clinical team could include that, as the retrospective crossmatch is historically positive and currently negative, antibody removal is not indicated. You could also recommend regular post-

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transplant monitoring (at 7 days post-transplant, 28 days post-transplant and quarterly thereafter for the first year or more frequently if clinically indicated) with a low threshold for intervention if AMR suspected.

Question 4
Recommendations for further testing: With the exception of the sample received 16/03/2020, the MFI levels for the donor directed beads is lower than the pre-transplant samples.
Also, the test for 16/03/2020 could be repeated. Is this a true increase as PC bead is also higher in this test than in the other samples?
It may also be beneficial to request a further sample from the patient to see current status and continue to monitor DSA post-transplant every 3 months or when clinically indicated

Follow up on the patient:
No post-transplant complications, currently shielding due to COVID-19 and doing well. Last DSA was a sample received 18/08/2020 where the MFI for the Cw1 directed bead was 1128.