UK NEQAS
International Quality Expertise

Histocompatibility & Immunogenetics

HNA-3a Antibodies - Laboratory Assessment and Immunological Risk

Amy De'Ath, Deborah Pritchard & Tracey Rees

Introduction

NEQAS provide an educational exercise to enable laboratories to compare results and clinical interpretations from multiple assays. Labs are asked to perform crossmatching, HLA typing and antibody detection/specification. The exercise is offered to participants annually with the aim of mimicking a renal transplant scenario.

There was one distribution of samples for this exercise in 2021 which comprised of one 'donor' blood sample and three 'patient' serum samples.

Methods

Laboratories were asked to perform the tests they routinely carried out in a live unrelated donor kidney transplant setting:

- HLA typing
- Antibody detection/specification
- Crossmatching

Participants were requested to list donor specific antibodies (DSAs) and to provide a clinical interpretation of the results envisaging that the three serum samples were from three different renal 'patients' who were all ABO compatible with the 'donor'.

42 labs participated in the 2021 distribution, 16 of which were based in the UK and Ireland (UK&I). Please note that not all users reported results on every aspect of the Scheme.

One serum used in the scheme was from a HNA-1b/1b female patient that had a confirmed HNA-1a antibody. This patient was sensitised by 3 pregnancies and multiple blood transfusions.

Results

Of the 16 UK&I labs testing this sera in the educational exercise;

- 100% concluded there were Class I IgG antibodies present
- 100% of labs detected no IgG Class II antibodies
- A positive flow cytometry crossmatch (FCXM) result was reported by 100% (16/16) labs for T–cells and 93% (14/15) for B-cells
- Complement Dependent XM (CDCXM) results were inconsistent
- Labs reported no HLA DSAs in this sample.

Labs were asked to provide an interpretation of this results. Comments included:

- Negative CDCXM
- Positive FCXM
- No detectable DSA but the patient does have HLA antibodies
- Possible HNA-3a antibody present
- Requires further investigation

When asked to rate the risk for this 'transplant'

- 5/15 (33%) labs classed this as a high risk/contraindication to transplant
- 1/15 (7%) classed it as intermediate risk
- 9/15 (60%) classed it as standard risk.

Interestingly, the BSHI BTS Guidelines for the Detection and Characterisation of Clinically Relevant Antibodies in Allotransplantation (2014) indicate that this would be classed as a standard risk transplant as reactivity is most likely due to non-HLA antibodies.

When participants were asked what their immunological advice would be they suggested performing further testing such as repeating the crossmatch and antibody screening, HNA genotyping, performing an autologous crossmatch, requesting further information on the patient such as disease status, sensitisation events and possible pharmacological interference, screening for non-HLA antibodies.

Discussion

HNA is found on platelets, lymphocytes, endothelial, kidney, spleen and placental cells. Approximately 5% of the local population are HNA-3b/3b and can become sensitised through exposure to HNA-3a.

There are limited published studies on the impact of HNA antibodies in transplantation although antibody mediated rejection and early graft loss were noted in a series of 7 UK case reports (Key *et al*., 2019). All patients were female, had an unexplained positive T and B cell FCXM with no HLA DSA defined. Retrospective testing identified they were all HNA-3b/3b with HNA-3a antibodies. Two of the patients had graft failures at 10 and 12 months.

A further two cases, from Edinburgh, were reported in an abstract for the EFI/BSHI conference held in 2021 (McConnell *et al*., 2020). In these cases one of the patients who received a live donor transplant has a functioning graft and no AMR, but the other patient who was the recipient of a deceased donor kidney had antibody mediated rejection and poor graft function despite treatment with ATG and rituximab.

HNA-3a antibodies are likely rare in transplant waiting list patients (Key *et al*., 2020 estimated 1%), but patients who develop them will be highly sensitised (approx. 95% of donors express HNA-3a).

In this educational scheme, the majority of laboratories reported a positive FCXM in the absence of HLA DSA. However, there was wide variation in the clinical risk associated with these results. It is not current practice to perform HNA typing/antibody screening; some labs suspected HNA-3a antibodies from the laboratory XM results, but they cannot be defined without additional specialist testing (currently only performed by 1 lab in the UK). HNA-3a antibodies will not be detected in a virtual XM.

Laboratories should be aware of the potential for these non-HLA antibodies to cause a strong positive XM in the absence of HLA DSAs, which may be associated with poorer transplant outcome. NEQAS distributed this serum to highlight this issue, especially for laboratories that may not have previously seen sera containing HNA-3a antibodies.

RESULTS	CDCXM						FCXM	
	PBL		T-Cells		B-Cells			
	Without-DTT	With-DTT	Without-DTT	With-DTT	Without-DTT	With-DTT	T-Cells	B-Cells
Consensus	*None*	Positive	Negative	Negative	*None*	*None*	Positive	Positive
Number Positive	2 (50%)	3 (75%)	1 (25%)	0 (0%)	3 (50%)	4 (67%)	16 (100%)	14 (93%)
Number Negative	2 (50%)	1 (25%)	3 (75%)	4 (100%)	3 (50%)	2 (33%)	0 (0%)	1 (7%)
HLA IgG Antibodies Present	Class I	Yes (100%)			Class II	No (100%)		
Risk	Contraindication/High	5 (33%)		Intermediate		1 (7%)	Standard	9 (60%)

Table 1: Summary of Crossmatch Results, HLA Antibody Detection and Interpreted Immunological Risk

Further Information

Full information on all UK NEQAS for H&I schemes is available at www.neqashandi.org.uk or contact **ukneqashandi@wales.nhs.uk**

References

Key T, Carter V, Day S, Goodwin J, Goodwin P, Knight A, Mather F, Poles A, Shaw O, Rigg K, McKane W Human Neutrophil Antibodies are Associated with Early and Chronic Antibody Mediated Rejection in Kidney Transplant Recipients.2019, J Renal Transplant Sci, 2(2), 81.

McConnell S, Battle R, Henderson L, Calvert C, Sellers F, Poles A, Turner D. Unexpected positive flow crossmatches due to the presence of HNA-3a antibodies. 2020. HLA, 95(4), 346.

GIG CYMRU NHS WALES
Ymddiriedolaeth GIG Felindre Velindre NHS Trust

WELSH TRANSPLANTATION AND IMMUNOGENETICS LABORATORY

Welsh Blood Service
Gwasanaeth Gwaed Cymru