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# Human Neutrophil Antibodies Associated with Early and Chronic Antibody Mediated Rejection in Kidney Transplant Recipients

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**Abbreviations:** ATG: Anti-Thymocyte Globulin; CDC: Complement Dependant Cytotoxicity; DBD: Donation After Brain Stem Death; DCD: Donation After Circulatory Death; DSA: Donor Specific Antibody; FCXM: Flow Cytometry Cross-match; GIFT: Granulocyte Immunofluorescence Test; HLA-Ab: Human Leucocyte Antigen specific Antibodies; HLA-DSA: Human Leucocyte Antigen Donor Specific Antibodies; HNA: Human Neutrophil Antigens; LIFT: Lymphocyte Immunofluorescence Test; LKD: Living Kidney Donor; NAIN: Neonatal Alloimmune Neutropenia; MICA: Major Histocompatibility Complex Class I Polypeptide-Related Sequence A; MICB: Major Histocompatibility Complex Class I Polypeptide-Related Sequence B; RMF: Relative Mean Fluorescence; TRALI: Transfusion Associated Acute Lung Injury

## INTRODUCTION

Antibodies directed against endothelial and non-Human Leucocyte Antigen (HLA) targets are known to account for positive cross-matches and cause graft dysfunction in recipients of kidney transplants both in the absence of HLA specific antibodies (HLA-Ab) and where no donor HLA specific antibodies (HLA-DSA) can be detected [1].

Non-HLA antigens to which antibodies have been detected include the Major Histocompatibility Complex class I polypeptide-related sequence A and B (MICA and MICB) [2], vimentin [3], angiotensin II type I receptor antibody [4] and glutathione-S-transferase [5]. These antigens are both polymorphic (MICA and MICB) and non-polymorphic (vimentin). Antibodies directed against these antigens have been reported to be associated with antibody mediated rejection in solid organ transplantation.

The Human Neutrophil Antigen (HNA) system currently consists of five identified groups (HNA 1-5), with limited polymorphism, present on diverse extracellular structures including Fc-γ receptor IIIb (HNA-1) and choline transporter-like protein 2 (HNA-3) [6]. HNA-3 is expressed on neutrophils, lymphocytes, platelets, endothelial cells, kidney and spleen. HNA-3 is a bi-allelic system comprising HNA-3a (SLC44A2*01) and 3b (SLC44A2*02) Alloimmunisation to HNA occurs mainly in pregnancy where the mother produces neutrophil specific antibodies to mismatched paternal antigens present in the foetus. Around 5% of the Caucasian population are homozygous for HNA-3b and thus at risk of allosensitisation to HNA-3a. HNA specific antibodies are known to be clinically relevant and are a cause of transfusion associated lung injury and neonatal alloimmune neutropenia [7,8]. The expression of HNA on lymphocytes has the potential to result in a positive

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kidney donor cross-match in allosensitised individuals where the donor carries the corresponding mismatched HNA.

## MATERIALS AND METHODS

Seven kidney transplant recipients with identified HNA-3a antibodies and transplanted between 2006 and 2018 were analysed as a series of case studies. All recipients were Caucasoid females, with a sensitisation history of pregnancy, pregnancy and blood transfusion or in one case a previous kidney transplant.

All HNA testing was performed retrospective to the transplant. All recipients and donors were HNA genotyped by sequence-based typing methods. Recipients were tested for HNA-Ab by indirect granulocyte immunofluorescence test (GIFT), lymphocyte immunofluorescence test (LIFT) utilising recombinant HNA-3a positive cell lines and Luminex®. Recipient HLA specific antibodies were detected and characterised using a combination of Luminex® based assays (One Lambda Inc LABScreen® single antigen, screen and I.D, analysed using a LABScan™ 100 flow analyser). Pre-transplant crossmatch was by 3 colour flow cytometry (FCXM) and in addition in case 2 complement dependant cytotoxicity (CDC). Positivity in the FCXM was determined by centre specific criteria and reported as relative mean fluorescence (RMF). Histological analysis was performed using the current Banff classification of renal allograft pathology in use at the time of the kidney transplant biopsy. Maintenance immunosuppression consisted of standard triple therapy using tacrolimus, mycophenolate mofetil and prednisolone. Induction therapy was with either anti-thymocyte globulin (ATG) or Basiliximab.

## RESULTS AND CASE REPORTS

The kidney transplants were performed at five different UK centres (2 of the centres each transplanted 2 recipients). Four of the seven recipients had at least one previous unexplained positive FCXM with other potential donors which had resulted in non-proceeding transplants (range 1-6). The donors included both living donors (LKD) and deceased donors (DCD and DBD). All recipients except patient 7 received induction therapy at the time of transplant due to the positive pre-transplant donor cross-match. Patient 7 was found to have a positive cross-match on retrospective testing following a virtual cross-match **(Table 1)**.

**Table 1.** Summary table of demographic and clinical history for the seven cases including patient gender, age, primary renal disease, year of transplant, type of donor, previous sensitisation history, the number of previous non-proceeding transplants and induction therapy regimen

| Patient | Gender | Age | Primary renal disease | Transplanted | Donor type | Sensitisation | Previous non-proceeding transplant | Induction therapy |
|---|---|---|---|---|---|---|---|---|
| 1 | F | 43 | Small kidneys cause unknown | 2006 | DBD | Pregnancy | 3 | ATG |
| 2 | F | 57 | ADPKD | 2013 | DCD | Pregnancy+ Transfusion | 1 | Basiliximab |
| 3 | F | 55 | ADPKD | 2014 | LKD | Pregnancy+ Transfusion | 6 | ATG |
| 4 | F | 37 | Cystinosis | 2015 | LKD | Transplant | 2 | Basiliximab |
| 5 | F | 60 | ADPKD | 2016 | DCD | Pregnancy+ Transfusion | 0 | Basiliximab |
| 6 | F | 61 | Renovascular disease | 2017 | DCD | Pregnancy | 0 | Basiliximab |
| 7 | F | 57 | ADPKD | 2018 | DBD | Pregnancy | 0 | Basiliximab |

HNA-3a specific IgG Ab was detected in the pre-transplant sera of all recipients. HNA genotyping by PCR-SBT showed all recipients were HNA-3b3b. All donors expressed HNA 3a either as a homozygous (HNA-3a3a) or heterozygous (HNA-3a/3b) trait. The pre-transplant FCXM was positive in all cases. All recipients were tested for HLA Ab and we demonstrated that there was no detectable HLA DSA in these cases to explain the positive FCXM. Early biopsies from cases 1, 2, 4, 5 and 7 were consistent with acute AMR. Early biopsies from case 3 did not show acute AMR but a later biopsy performed to investigate heavy proteinuria and a falling eGFR showed CTG, a finding consistent with chronic AMR. Patients 2 and 5 suffered graft loss within 1 year. In the case of patient 2 delayed graft function triggered transplant biopsies showing vascular and cellular rejection at 5 days and persistent vascular rejection and positive C4d staining at 13 days. She received ATG and 5 sessions of plasma exchange over 2 weeks. A biopsy taken 25 days post-transplant showed healing vascular rejection and the patient was dialysis independent 8 weeks post-transplant. The patient lost their graft 1year post-transplant, resumed dialysis and died 3 years later. Patient 5's transplant was slow to function, and a transplant biopsy was performed on day 5 which showed acute antibody mediated rejection. A follow-up biopsy was performed one month later, and the

appearances were improving. However, nine months post-transplant her renal function started to deteriorate, and a further biopsy showed active chronic antibody mediated rejection and subsequently she returned to dialysis one month later **(Table 2)**.

**Table 2.** Summary table of donor HLA mismatch grade and HNA-3 genotype, pre- transplant flow cytometric cross-match relative mean channel fluorescence (RMF) value, presence or absence of antibody mediated rejection (ABMR) including histological findings and presence or absence of graft loss.

| Patient | HLA-A B DR mm | Donor HNA-3 | FCxm T cell RMF | FCxm B cell RMF | ABMR | Histology | Graft loss |
|---|---|---|---|---|---|---|---|
| 1 | 1.1.0 | 3a/3a | 9.0 | 4.2 | Y | Banff 2, acute antibody mediated rejection | N |
| 2 | 1.1.0 | 3a/3b | 6.4 | 5.6 | Y | Banff 4 IIa | Y |
| 3 | 0.1.0 | 3a/3a | 10.1 | 3.4 | Y | Banff 2, chronic antibody mediated rejection | N |
| 4 | 0.0.1 | 3a/3b | 4.0 | 4.0 | Y | Banff 2, acute antibody mediated rejection | N |
| 5 | 1.1.1 | 3a/3a | 4.0 | 4.0 | Y | Banff 2, acute antibody mediated rejection | Y |
| 6 | 1.1.1 | 3a/3b | 4.3 | 2.0 | N | No post-transplant dysfunction: No biopsy | N |
| 7 | 0.0.0 | 3a/3a | 2.7 | 3.2 | Y | Banff 2, acute antibody mediated rejection | N |

## DISCUSSION

A range of non-HLA proteins have been documented to be potential targets for antibodies in the setting of solid organ transplantation. Not all are expressed on the surface of leucocytes and hence may remain undetected in the context of the usual cross-match assays for transplantation. They can be identified in some cases using endothelial cell cross-match techniques and solid phase assays. Some are however expressed on leucocytes and therefore, may give rise to an unexplained positive cross-match in patients where there is no HLA specific antibody present or no HLA DSA. Where auto reactive antibodies are not present the laboratory should seek to identify the cause of the positive cross-match.

This report on seven individuals includes the first reported case (patient 2) of HNA-3a specific antibodies retrospectively detected in an individual with delayed graft function, vascular and cellular rejection at 5 days and persistent vascular rejection and positive C4d staining at 13 days. Following treatment, the patient did become dialysis independent but ultimately lost their graft after 1 year [9]. In the cases presented here all patients had a positive FCXM on the day of transplant with their respective kidney donors. We retrospectively investigated possible causes of this and found all the patients had circulating antibodies to HNA-3a. Antibodies to HNA-3a are the likely cause of the positive FCXM in these allosensitized individuals who are homozygous for HNA-3b. Such patients potentially face long waiting times for transplantation because around 95% of donors will express HNA-3a and these recipients are therefore likely to have multiple positive FCXMs which can lead to a decision to not proceed with the transplant. The high frequency of HNA-3a expressing individuals also accounts for the observed phenomena of previous positive cross-matches often with multiple potential apparently HLA compatible donors.

In this series of cases we report a range of outcomes although in only one case (patient 6) did the post-transplant course appears to be incident free. In the cases of patient 2 and 5 both grafts were lost within a year of transplant following deterioration in renal function. Patients 1, 3 and 4 and 7 had evidence of graft dysfunction including episodes of ABMR, although in these cases the kidneys are still functioning at 11 years, 3 years and 15 months and one-month post-transplant. It should be noted despite induction therapy four patients had severe early ABMR and one has chronic transplant glomerulopathy.

We estimate that the frequency of patients with HNA antibodies on a transplant waiting list would be less than 1%. The incidence of HNA-3a specific antibodies in a female donor population has been reported to be 0.26%; this was

defined whilst screening apheresis donors as part of the TRALI reduction programme in the UK [10].

The role for HNA-3a antibody testing is initially likely to be of most value in cases of non-HLA/unexplained positive FCXM. In the LKD setting there is clearly an appropriate time frame to investigate, whereas in the deceased donor setting this would not be currently possible. The frequency of HNA incompatible donors in these scenarios means a patient sensitised to HNA-3a is very unlikely to be offered a HNA compatible deceased donor kidney. One option maybe to investigate family members particularly siblings for a LKD with HNA compatibility. Where no realistic choice of a compatible HNA donor exists then pre-transplant antibody removal and augmented immunosuppression should be carefully considered. Further investigation is required to assess the pathological significance and prevalence of HNA antibodies in kidney transplantation. However, of note two patients with HNA-3a specific antibodies lost their transplanted kidney within a year.

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