A case of neonatal alloimmune neutropenia due to anti-HNA-4a antibodies

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Introduction

Neonatal alloimmune neutropenia (NAIN) occurs when maternal antibodies are produced against paternally derived human neutrophil antigens (HNA) present on foetal neutrophils that are incompatible with the maternal HNA phenotype. These antibodies can cause moderate to severe neutropenia in the new born. Neonates present with a range of symptoms from asymptomatic neutropenia to sepsis.

Case History

In this case, baby was born at 35^{+1} via emergency caesarean section due to breech presentation and was taken to the neonatal unit. Baby was started on intravenous antibiotics and bloods taken. It was noted that the baby was neutropenic which was confirmed on repeat samples. The counts remained low for the following days before returning to normal levels at day 6 (sample date 08/09/2021, Table 1).

Methods

Samples from mother, father and baby were referred to NHS Blood and Transplant for investigation of NAIN. All family members were genotyped for the HNA-1, -3, -4 and -5 by polymerase chain reaction sequence based typing (PCR-SBT) using in-house primers. Maternal serum was tested for the presence of neutrophil alloantibodies using the granulocyte immunofluorescence test (GIFT), granulocyte chemiluminescence test (GCLT), HNA Luminex bead assay (LabscreenMULTI) and Monoclonal Antibody Immobilisation of Granulocyte Antigens (MAIGA).

Table 1. Patient neutrophil count

Sample Date	Neutrophil Count
02/09/2019	0.3
02/09/2019	0.26
03/09/2019	0.74
04/09/2019	0.85
08/09/2019	4.24

Table 2: Reactivity of maternal serum in the GCLT and GIFT against HNA typed donor panel cells

	HNA Interpreted Epitope														
Donor	1a	1b	1c	1d	2	3a	3b	4a	4b	5a	5b	**GCLT**		**GIFT**	
MP	+	-	-	-	+	+	+	+	-	+	-	60.8	+	15.0	+
AD	-	+	-	+	+	+	-	+	-	+	+	55.2	+	10.7	+
GS	+	-	-	-	+	+	-	+	-	+	+	58.8	+	4.7	+
AP	-	+	-	+	+	+	-	+	-	+	-	82.1	+	18.8	+
NW	-	+	-	+	+	+	-	-	+	+	-	0.9	-	0.6	-

GCLT opsonic index ratio = Positive > 2 , GIFT median channel fluorescence ratio = Positive > 1

Results

Strong clinical evidence of NAIN was supported by the laboratory results . Serological testing by GIFT and GCLT suggested the presence of HNA-4a antibodies in the maternal serum (Table 2). These results were confirmed by HNA Luminex bead assay and MAIGA (Table 3 and 4). HNA genotyping of the family further support the presence of HNA-4a antibodies as incompatibilities were identified in HNA-1, -3, -4 and -5 between the mother and father (Table 5). However, only the HNA-1 and -4 incompatibilities were inherited by the baby. HNA-1a antibodies were excluded by Luminex and MAIGA.

Table 3. Reactivity of maternal serum in the HNA Luminex bead assay

Bead	Spec	Result	Raw Data	Ratio	Count
026	HNA-1a	Negative	292.27	0.93	136
027	HNA-1b	Negative	111.55	0.00	125
028	HNA-1c	Negative	136.02	0.11	106
029	HNA-2	Negative	200.86	1.41	112
031	HNA-3a	Negative	553.63	3.71	100
032	HNA-3b	Negative	535.91	2.27	122
033	HNA-4a	Positive	11949.01	236.37	154
034	HNA-5a	Negative	172.93	0.85	114
036	HNA-5b	Negative	294.26	2.66	135

Spec = HNA specificity detected

Table 4. Reactivity of maternal serum in the MAIGA assay

Donor ID	Donor HNA Type	MAb Specificity	MAb Clone	OD Negative control	OD Patient Serum (Positive OD>0.2)	
PB	4a4a	CD11b	Bear-1	0.034	0.703	+
NW	4b4b	CD11b	Bear-1	0.062	0.118	-
PB	4a4a	CD18	7E4	0.049	0.596	+
NW	4b4b	CD18	7E4	0.075	0.107	-
IM	1a1a	CD16	3G8	0.041	0.049	-
NW	1b1b	CD16	3G8	0.049	0.080	-
IM	1a1a	CD16	LNK16	0.052	0.059	-
NW	1b1b	CD16	LNK16	0.060	0.087	-

MAb = monoclonal antibody, OD =optical density

Table 5. HNA genotypes of the family

	HNA genotype	Interpreted Epitope
Mother	*FCGR3B**02; *SLC44A2**01; *ITGAM**02; *ITGAL**01	1a-, 1b+, 1c-, 1d+; 3a+, 3b-; 4a-, 4b+; 5a+, 5bw-
Father	*FCGR3B**01, *FCGR3B**02; *SLC44A2**01, *SLC44A2**02; *ITGAM**01, *ITGAM**02; *ITGAL**01, *ITGAL**02	1a+, 1b+, 1c-, 1d+; 3a+, 3b+; 4a+, 4b+; 5a+, 5bw+
Baby	*FCGR3B**01, *FCGR3B**02; *SLC44A2**01; *ITGAM**01, *ITGAM**02; *ITGAL**01	1a+, 1b+, 1c-, 1d+; 3a+, 3b-; 4a+, 4b+; 5a+, 5bw-

Discussion

Although the majority of reported NAIN cases are due to antibodies against HNA-1, -2 and -3, this case illustrates the importance of including testing using donor cells representing all HNA types especially where incompatibilities have been identified within the family.