

NEONATAL ALLOIMMUNE NEUTROPENIA DUE TO ANTI-HNA-1b

Iman Al-Sheikh, MD; Mohammed Al-Khalifa, MD;
Amjad Rahi, MD; Fakhira Al Qahtani MD

Neonatal alloimmune neutropenia (NAN) is an uncommon cause of neutropenia in the neonatal period. It is caused by the transfer of maternal alloantibodies across the placenta and their binding to specific antigens on the baby's neutrophils. Affected newborns present with bacterial infections which can be serious. The distinction between NAN and other causes of neutropenia during this age group, such as congenital neutropenia or neutropenia of sepsis, may be clinically difficult to establish. Diagnosis usually requires the demonstration of neutrophil alloantibodies in maternal serum reacting with neutrophils from the newborn and the father but not from the mother. Difficulties sometimes arise in both direct and indirect antihuman globulin (AHG) tests due to nonspecific adsorption of AHG reagent by receptors to the constant fragment of antibody. Besides, harvesting sufficient neutrophils from severely neutropenic samples may be difficult. Ideally, one should also demonstrate that the maternal antibody specificity is directed towards an alloantigen expressed on newborn's neutrophils but not on maternal cells. This requires the need to identify the neutrophil alloantibody and to type the family for human neutrophil antigen (HNA). The former has recently become possible by serological techniques that are not widely available, such as monoclonal antibody immobilization of granulocyte antigens (MAIGA) test.

Typing neutrophil antigens used to rely on serological methods which suffer from a number of limitations. The recent application of molecular technology to this field has enabled direct genotyping of neutrophil genes. This is a major breakthrough since it enables diagnosis from any cell in the body as well as facilitating prenatal diagnosis.

Among the many antigens expressed on the surface of the neutrophil, a few are specific to this cell. These neutrophil-specific antigens were recently reclassified into

immunized and produce specific antibodies once exposed to the foreign antigen through transfusion or pregnancy.

NAN has been reported to occur due to antibodies directed against HNA-1 antigens *a b* or *c*, the most common being anti-HNA-1a.^{2,3} Rare mothers with HNA-1 null phenotype have been reported to produce isoantibodies that result in NAN.⁴ The clinical contribution of the different HNA antibodies to NAN depends, to a large extent, on the local frequency distribution of HNA antigens. Recently, we reported the frequency distribution of HNA-1(a,b) antigens in Saudis.⁵

The objective of this study was to establish the basis of diagnosis of NAN in a neonate. To our knowledge, no previous case of NAN due to an identifiable neutrophil antigen system has been reported in the Kingdom.

Case Report

A 14-day-old female Saudi newborn who was the product of full-term pregnancy and normal vaginal delivery presented to the pediatric emergency room with umbilical sepsis. The mother had noticed that the baby's feeding was suboptimal. The newborn had been receiving breast milk since birth. She was the fifth child and there was no family history of recurrent infections (Figure 1). Physical examination revealed an active baby not in distress, with a body temperature of 38°C, and umbilical swelling with purulent discharge. Other examinations were unremarkable.

Indirect Granulocyte Immunofluorescent Test (GIFT)

WBC and neutrophil counts were obtained by automated counting on the Coulter S Plus IV system. Granulocytes from the father's blood sample were separated by Ficoll-Hypaque system. A unit of 0.1 mL of separated granulocytes were incubated with 0.1 mL of the mother's serum at 37°C for 30 minutes (Figure 2). Cells were then washed three times with PBS/BSA, and finally the supernatant was decanted. Fluorescent-labeled AHG

From the Departments of Pathology and Pediatrics (Drs. Al-Sheikh and Al Qahtani), King Faisal University, and the Department of Immunology (Drs. Rahi and Al-Khalifa), Regional Laboratory and Blood Bank, Dammam, Saudi Arabia.

Address reprint requests and correspondence to Dr. Al-Sheikh: Department of Pathology, King Faisal University, P.O. Box 30500, Al-Khobar 31952, Saudi Arabia.

Accepted for publication 15 April 2002. Received 25 August 2001. the HNA system.¹ Previously, the same antigens were called neutrophil antigens (NA), Fcγ receptors (FcγRIIIb), or CD16 molecules. They are physiologically important in the clearance of antigen antibody complexes. Individuals who are negative for a certain HNA allele may become

FIGURE 1. Family history of neonatal alloimmune neutropenia.

FIGURE 2. Indirect granulocyte immunofluorescent test (GIFT).

FIGURE 3. Agrose gel electrophoretogram of family.

was added and mixed with cell button. The mixture was incubated at room temperature for 30 minutes in the dark. The mixture was again washed in PBS/BSA twice and the

supernatant was decanted. The cell button was mixed with 0.5 mL of glycerol-PBS and mounted on a cover slip. The slide was examined using 40x objective and epifluorescent UV illumination.

HNA Genotyping

DNA was extracted by the salting out technique with ethanol precipitation. Extracted DNA was amplified in a Perkin Elmer Gene Amp 9600 using PCR-SSP technique as described earlier.^{5,6} The amplified product was detected by electrophoresis on 3% agarose gel with ethidium bromide staining. Amplified products of HNA-1a and HNA-1b were recognized based on the probe being added and on the MW size, the former being 53bp shorter.

Results

Laboratory investigations revealed isolated neutropenia, total WBC count was $7.9 \times 10^3/\mu\text{l}$, with 2% neutrophils, making an absolute neutrophil count of 158 μl . The remaining blood counts were within normal range. Indirect GIFT was performed on maternal serum incubated with paternal neutrophils. The result was strongly positive but was weakly positive with control serum. We had difficulty in interpreting the GIFT result. HNA-1a and HNA-1b were genotype in the father, mother and newborn. The father and the baby were found to be heterozygotes HNA-1(a+b-) (Figure 3). The mother typed homozygous HNA-1(a+b-), Table (1).

The newborn was put on antibiotic treatment and her clinical condition improved after one week. Neutrophil count was followed-up and was found to normalize spontaneously at the age of 40 days. Total WBC count was $12.6 \times 10^3/\mu\text{l}$, 72% of which were neutrophils, producing an absolute neutrophil count of 9072/ μl .

Discussion

We diagnosed this case as NAN based on clinical exclusion of other causes of neutropenia and based on laboratory findings. Spontaneous recovery of neutropenia also supported our clinical impression. In our opinion, the positive result with GIFT in the control sample was possibly due to nonspecific binding of the fluorescent-labeled AHG reagent to FC receptors on neutrophils (Figure 2). The use of the antibody fragment of AHG reagent would eliminate such difficulty. Nevertheless, the difference in extent of fluorescence between the control and test samples suggested true positive result.

HNA genotyping revealed no polymorphism of HNA-1a antigen in the family but HNA-1b was polymorphic. Both father and baby expressed HNA-1b genes but the mother

Mother	+	-	HNA-1(a+, b-)
Father	+	+	HNA-1(a+, b+)
Baby	+	+	HNA-1(a+, b+)

was negative for this allele. We speculated that the antineutrophil antibody was directed against HNA-1b antigen as this system was shown in previous studies to be highly polymorphic and only second to HNA-1a in its clinical correlation with NAN. We did not have direct means of identifying maternal antibody as MAIGA test is not available in our laboratory. We also could not type the entire HNA antigen because techniques for full HNA typing outside HNA-1 system are limited worldwide at the time of the study. This newborn was the fifth child, and none of her siblings had a similar history of infections during the neonatal period. Negative family history may be explained by the fact that previous siblings may not have been affected because they were homozygous HNA-1(a+b-) or that they were mildly subclinically affected. We concluded that future pregnancies by the mother may be similarly affected. In fact, the probability of the neonate having another affected sibling is 50% and this possibility was discussed with the parents.

To our knowledge, this is the first reported case of NAN due to anti HNA-1b antibody in Saudi Arabia. Availability of laboratory facilities that positively support the clinical impression is recommended.

Acknowledgements

We acknowledge the help of Mrs. Asha Paul who assisted in typing this manuscript.

References

1. Bux J. Nomenclature of granulocyte alloantigens. ISBT Working Party on Platelet and Granulocyte Serology, Granulocyte Antigen Working Party. International Society of Blood Transfusion. *transfusion* 1999;39:662-3.
2. Bux J, Jung KD, Kauth T, Muller-Eckhardt C. Serological and clinical aspects of granulocyte antibodies leading to alloimmune neonatal neutropenia. *Transfusion Med* 1992;2:143-9.
3. McCullough J, Clay ME, Stroneck DE. Granulocyte allogeneic systems and their clinical significance. In: Nance ST, editor. *Alloimmunity*. Bethesda: American Association of Blood Bank, 1993:49-82.
4. Pug N, DeHas M, Kleijer M, et al., Isoimmune neonatal neutropenia caused by Fc γ RIIIb antibodies in a Spanish child. *Transfusion* 1995;35:n 683-7.
5. Al-Sheikh I, Al Khalifa M, Rahi A. Frequency distribution of neutrophil alloantigen in Saudis. PCR-SSP study of 100 healthy Saudi male blood donors. *Saudi Med J* 2002 ;5:548-51.
6. Hessner MJ, Curtis BR, Endean DJ, Aster RH. Determination of neutrophil antigen gene frequencies in five ethnic groups by polymerase chain reaction sequence-specific primers. *Transfusion* 1996;36:895-9.

TABLE 1. Results of HNA-a genotyping in the family.

	Amplification		Genotype
	HNA-1a	HNA-1b	
Mother	+	-	HNA-1(a+, b-)
Father	+	+	HNA-1(a+, b+)
Baby	+	+	HNA-1(a+, b+)