Neonatal Alloimmune Neutropenia

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Keywords

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Summary

Neonatal alloimmune neutropenia (NAIN, NAIN or NIN) is a neutrophil blood group antagonism, analogous to hemolytic disease of the fetus and newborn (HDFN) and fetal/neonatal alloimmune thrombocytopenia (FNAIT). A limited number of prospective screening studies showed that granulocyte-specific antibodies were detectable in 0.35–1.1% of random postnatal maternal samples and that the incidence of NAIN was below 0.1%. Symptoms vary from none to mild skin infections, omphalitis or more severe infections like pneumonia, sepsis, and meningitis. Treatment of neonatal infection with antibiotics and granulocyte-colony stimulating factor is advised.

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Introduction

After several reports [1, 2] of siblings suspected for immune mediated agranulocytosis in the 1950s, it was in 1960 that Lalezari et al. [3] first proved the presence of maternal antibodies causing severe neutropenia (complete absence of granulocytes) in three of four siblings and named the disorder ‘isoimmune neonatal neutropenia’. Unfortunately, the first child died of pneumonia. After an unaffected second child, the third child developed severe scalp inflammation and possibly sepsis 8 days postpartum. She was treated with antibiotics, and her neutrophil count normalized approximately 70 days postpartum. The fourth child developed omphalitis

8 days postpartum, which was treated with antibiotics. Her neutrophil count did not respond to corticosteroids, but normalized after 71 days. Total leukocyte counts in the children were normal, as the severe neutropenia was compensated by a monocytosis.

Already this early report very well characterizes neonatal alloimmune neutropenia [3]. In this disease, the mother has developed alloantibodies against a certain neutrophil antigen, which the fetus inherits from the father. In this particular case, the father had been heterozygous for the antigen in question, explaining the unaffected second child. Shortly after this report, the first detected neutrophil antigens were described and named NA1, NA2 and NB1 [4, 5]. Analogous to the PLA nomenclature for platelet antigens, proposed by Shulman, the name was composed of the N, for neutrophil specificity, the A, B etc. for the order the antigen system (genetic locus) was detected, and the numbers to identify alleles [4]. In 1998, this nomenclature was changed to the currently used human neutrophil antigen (HNA) system and updated in 2015 [6, 7]. Currently, 11 HNAs within five different HNA systems are known: four HNA-1 alleles, HNA-2, two HNA-3, two HNA-4 alleles, and two HNA-5 alleles (table 1) [7]. For the detection of the anti-neutrophil antibodies, Lalezari and Spaet [8] used the leukoagglutination technique. This technique is still being used, especially for the detection of anti-HNA-3 antibodies [9]. However, in the 1970s and 1980s the granulocyte immunofluorescence test (GIFT) [10] and monoclonal antibody immobilization of granulocyte antigens (MAIGA) [11] became available, and recently bead-based antibody detection methods have been developed [12, 13].

There may be some confusion about which name can best be used for this condition. We decided to use in this review the name fetal/neonatal alloimmune neutropenia (FNAIN), analogous to fetal/neonatal alloimmune thrombocytopenia (FNAIT), which is commonly used for the platelet antagonism, and not isoimmune neonatal neutropenia. Furthermore, in this paper we shortened FNAIN to neonatal alloimmune neutropenia (NAIN), as it is actually unknown if already the fetal neutrophil count is affected and

Table 1. The HNA antigen system, consisting of five systems carrying 11 HNAs and one variant HNA

Antigen system	Carrier glycoprotein	CD	Antigen	Allele	Variant	Allele frequency Caucasians	Allele frequency Asians	Allele frequency Africans
HNA-1	FcγRIIIb	CD16b	HNA-1a HNA-1b1d HNA-1b1c	FCGR3B*01 FCGR3B*02 FCGR3B*03		0.28–0.39 0.57–0.71 0.014–0.03	0.30–0.78 0.22–0.70 0	0.35–0.42 0.35–0.57 0
FcγRIIIb deficient	FCGR3B*02 244A>G		HNA-1a HNA-1b var.	FCGR3B*04 FCGR3B*05 gene deletion	FCGR3B*01 316G>A FCGR3B*02 244A>G	0.15%	<1%	<1%
HNA-2 CD177 deficient	CD177 glycoprotein	CD177	HNA-2	CD177*01 mRNA splicing		97% [†] 3% [†]	88–99% 1–12%	
HNA-3	CTIL2		HNA-3a HNA-3b	SLC44A2*01 SLC44A2*02		0.79 0.21	0.49–0.74 0.26–0.51	0.97 0.03
HNA-4	MAC-1; CR3; αβ2-integrin	CD11b	HNA-4a HNA-4b	ITGAM*01 ITGAM*02		0.9 0.1	0.88–1.0 0–0.03	0.89 0.11
HNA-5	LFA-1; αLβ2-integrin	CD11a	HNA-5a	ITGAL*01 ITGAL*02		0.66 0.34	0.79–0.96 0.04–0.35	0.5 0.5
Allele frequencies (antigen percentages) depend on the specific population, especially for the Asian frequencies. Only limited numbers of African individuals are typed [20–22].								

because the clinical symptoms are, in contrast to FNAIT, not already present in the fetus. Neonatal immune neutropenia (NIN) is mostly due to maternal alloantibodies directed to the non-self-fetal (paternal) neutrophil-specific antigens. Rarely, in cases of maternal autoimmune neutropenia, maternal neutrophil specific autoantibodies of the IgG class can cause neonatal neutropenia [14]. In this overview we therefore, focus on the NAIN.

Pathology

NAIN results from maternal sensitization to paternal HNAs present on the fetal neutrophils. The anti-HNA antibodies of the IgG immunoglobulin class are transported across the placenta and bind to the fetal neutrophils. It is unknown whether the antibodies only lead to increased destruction of mature cells or also to inhibition of granulocytopoiesis. In some cases NAIN is caused by isoantibodies, if a mother is lacking a complete HNA system-carrying structure (e.g. anti-FcγRIIIb isoantibodies or HNA-2 antibodies) [15].

It seems that NAIN can already occur in first pregnancies. In the published series, percentages for NAIN in first pregnancies vary between 5 and 40% [4, 15, 16]. This implies that maternal immunization can take place during pregnancy or even before the first pregnancy. In our healthy blood donor screening study, we detected anti-FcγRIIIb antibodies and anti-HNA-1a antibodies of the IgG class in 0.8% of never allo-exposed (meaning never transfused, organ-transplanted or pregnant) women [17, 18]. In this study, in healthy blood donors, we did not perform an HNA typing, and, therefore, we do not know if the detected antibodies were auto- or alloantibodies [17, 18]. Further investigation will be necessary to explain the presence of granulocyte-specific antibodies in never allo-exposed women and primiparous women. Expression of the human platelet antigen 1a (HPA-1a) on glycoprotein αvβ3 on placental endothelial cells is one of the reasons for alloimmunization against HPA-1a already in the first pregnancy [19]. However, most of the involved granulocyte antigens are not expressed on placental tissue, and therefore, only exposure of these antigens on fetal granulocytes seems to be the immunizing trigger during pregnancy. In table 1, the currently known HNAs and allele frequencies for Caucasians are shown. The allele frequencies vary for Asians, depending on the studied population group. One remarkable difference compared with the Caucasian population is the complete absence of the HNA-1c allele. Only a very limited number of studies have been performed on the allele frequencies in the African population [20–22].

The HNA-1 system is carried by the FcγgammaReceptor IIIb (FcγRIIIb) and includes four HNAs: HNA-1a (*FCGR3B*01*), HNA-1b, HNA-1c, and HNA-1d. HNA-1c and HNA-1d can only be present in combination with HNA-1b, i.e. HNA-1b1c (*FCGR3B*03*) or HNA-1b1d (*FCGR3B*02*). Recently the *FCGR3B*01* 316G>A and the *FCGR3B*02* 244A>G variants were found and named *FCGR3B*04* and *FCGR3B*05*, respectively (table 1) [7, 22–26]. Variation in expression of the HNA-1 alleles is

possible. About 0.1% of the population, regardless of which origin, is deficient for FcγRIIIb, resulting in the HNA-1null phenotype. These individuals can be immunized and produce anti-FcγRIIIb isoantibodies [27]. Furthermore, individuals can, as a result of gene duplication, have a higher expression of FcγRIIIb and subsequently be positive for more than two HNA-1 alleles [28–31]. Individuals who are HNA-2-positive mostly also have a CD177(HNA-2)-negative neutrophil subpopulation, due to lack of gene transcription in a subset of the cells [5, 32, 33]. This CD177-negative subpopulation can vary between almost 0% and almost 100%. HNA-2-negative individuals do not express CD177, as a result of an incorrect splicing process generating premature stop codons and can be immunized against HNA-2 [33–35]. The biallelic HNA-3 system is located on the choline transporter-like protein 2 (CTL2) and includes the HNA-3a and HNA-3b alleles [36, 37]. HNA-4 and HNA-5 are located on the αM subunit (CD11b) and αL (CD11a) of the αMb2 and αLb2 integrins, respectively. HNA-4a, HNA-4b, and HNA-5a result from single nucleotide polymorphisms [38–41]. Maternal sensitization to HNA-1a to HNA-1d, FcγRIIIb, HNA-2, HNA-3a, HNA-3b, HNA-4a, HNA-4b, and HNA-5a leading to NAIN have all been reported. Most cases are caused by antibodies specific for the FcγRIIIb located antigens HNA-1a and HNA-1b, followed by anti-HNA-2 and anti-FcγRIIIb [3, 15, 16]. The other antibody specificities are only rarely detected. Cases of NAIN due to maternal anti-HNA-1c, anti-HNA-1d, anti-HNA-3a, anti-HNA-3b, anti-HNA-4b, and anti-HNA-5a antibodies have been described in rare case reports [16, 24, 25, 42–45]. Antibodies against HNA-5b have never been detected.

Incidence

The incidence of NAIN is not exactly known. Due to the necessary laborious anti-HNA antibody screening and identification as says the known screening studies are limited in size. Furthermore, during the past decades different granulocyte-specific antibody detection techniques were used, and only the antibodies specific for the, at the time of the studies, known HNAs could be identified. It is therefore possible that some antibodies were missed due to incomplete antibody identification panels.

Bux et al. [15] detected 11 (1.1%) granulocyte-specific antibodies in 1,016 unselected samples postnatally drawn. Four (0.4%) of these 11 antibodies, were allogeneic and specific for one of the known HNAs. Zupanska et al. [46] genotyped 1,038 unselected women who had given birth for HNA-1a and HNA-1b and subsequently genotyped the newborns of 490 HNA-1a or HNA-1b homozygous women. Finally they performed an HNA-1 antibody screening for 195 of 203 women who delivered incompatible newborns and detected nine granulocyte-reactive (non-HLA) antibodies (0.9%), six anti-HNA-1a or HNA-1b and three antibodies with unknown specificity. Interestingly, in both above mentioned studies, none of the newborns delivered by mothers with granulocyte-specific antibodies had signs of infection or neutrophil counts below $1.5 \times 10^9/l$. Han et al. [47] detected three

NAIN cases in 856 neonates (0.35%) admitted to neonatal intensive care units in Korea.

In an HLA- and granulocyte-specific antibody screening, we detected specific neutrophil antibodies in 27 of 2,268 (1.2%) healthy female blood donors [17, 18]. Nine (0.8%) of these antibodies, directed against FcγRIIIb (n = 5) and HNA-1a (n = 4), were detected in 1,109 nulliparous never allo-exposed women and 18 (1.6%), directed against FcγRIIIb (n = 3), HNA-1a (n = 6), HNA-1b (n = 3), HNA-2 (n = 2) and HNA-3a (n = 4), in non-transfused primiparous or multiparous women. We did not type the women, and it is likely that the specific neutrophil antibodies, especially in never allo-exposed women, are (partly) autoantibodies, as it is known that neutrophil autoantibodies can be specific for FcγRIIIb and HNA-1a. Furthermore, most pregnancies were already way back longer than 1 year before drawing the blood samples, and antibody levels possibly decreased under the detection levels.

In clinical practice, requests for serological investigation for suspected NAIN for only one in 37,165 newborns are sent to our Sanquin reference laboratory being the only granulocyte serology laboratory in the Netherlands, and NAIN was only diagnosed in one of 118,929 newborns. In our study, this equated to 35 cases over a period of 22.5 years, with approximately 185,000 newborns during each year of the study period [16]. There are a number of explanations for this extremely low detection rate. Firstly, many NAIN cases do not show any symptoms, including the 14 (40%) of the neonates in our series who did not have any signs of infections but had neutropenia. Secondly, there are other possible causes of neutropenia that make NAIN harder to detect. Thirdly, clinicians may not be aware of the necessity of serological investigations or may consider it unnecessary to perform them. It is advised to diagnose NAIN in order to choose the best prophylactic and/or therapeutic treatment for the neutropenic neonate, to predict the clinical cause, and to anticipate on fetal/neonatal neutropenia in any subsequent children.

Symptoms

A reduced ANC is mostly already present at birth, but may decrease in the first week post-partum and can last for approximately six months. Most diagnosed NAIN cases, being the cases with clinical symptoms, showed ANCs below 500/μl. A high incidence of miscarriage was reported by Lalezari et al. [4] (19%) and Bux et al. (50%) [15]. In our series, ‘only’ 2 (6%) of 35 women experienced a miscarriage [16]. It is very likely that in most cases of NAIN (i.e. neutropenia due to maternal neutrophil antibodies) the neonates do not present with infection and/or other clinical symptoms. As already mentioned above, also in our series 14 (40%) of the 35 NAIN cases were detected because cell counts and differentiations were performed for other, non-related, disorders [16]. If symptoms are present, they vary from omphalitis, fever of unknown origin to more severe infections like pneumonia and sepsis [3, 15, 16]. It must be realized that NAIN, causing fulminant infections, can be a potentially fatal condition. Several case reports about severe sepsis

or meningitis as a result of NAIN have been published [15, 48–50]. As was described in the report of Lalezari et al. [3], infections are not always immediately present, but often start several days after birth. Any early effects of absolute neutrophil count (ANC) on the unborn child have not yet been sufficiently investigated.

Diagnosis

Neutrophil concentrations for neonates vary considerably in the first week postpartum, with the highest level 6–12 h postpartum [51, 52]. The concentrations are partly depending on the gestational age, birth weight, gender, and race. Normal blood neutrophil counts for newborns with a birth weight > 1500 g in the first week vary between 2,000 and 14,500/μl [51]. Normal blood neutrophil counts for a newborn with a birth weight < 1500 g can be as low as 500/μl [53]. Schmutz et al. [52] found cord blood ANC in 30,354 neonates, ranging between 2,700 and 13,000/μl for infants born >36 weeks gestation, between 1,000 and 12,500/μl for infants born at 28–36 weeks gestation and between 1,300 and 15,300/μl for infants born at <28 weeks gestation. This was measured in a population where ethnic or environmental factors may have been of influence.

The varying neutrophil concentrations may give rise to doubt about when further investigation is needed. Furthermore, ANCs < 1,000/μl is frequently seen at neonatal intensive care units, especially in preterm infants. Neutropenia in a neonate is mostly due to non-immune causes including sepsis, low birth weight, and pregnancy-induced maternal hypertension (table 2) [54–56]. Clinicians should consider further evaluation of neonatal neutropenia if no clear underlying cause is present and/or if the ANC does not increase within 3–5 days. Severe neutropenia (<500/μl) persisting more than 2 days requires additional evaluation [54]. If no maternal granulocyte-reactive antibodies are detected, congenital neutropenia might be present. Bone marrow examination and treatment with recombinant granulocyte-colony stimulating factor (G-CSF) should then be considered [56].

Serological investigation for cases suspected of NAIN is laborious and needs the availability of HNA-typed donors, equipment and reagents, HNA (geno)typing facilities and skilled experienced technicians. The standard serological work-up for NAIN includes: maternal HNA antibody detection and identification, HNA (geno)typing of both parents and neonate. A cross-match performed with maternal serum and paternal granulocytes is highly recommended.

Several different neutrophil antibody detection/identification methods, of which only the recently developed Luminex beads assays are commercially available, are used [9–13]. It is recommended by the ISBT Working Party on Granulocyte Immunobiology to use the GIFT in combination with the granulocyte agglutination test (GAT) [57]. If possible, the HNA specificity should be confirmed with an antigen-specific assay, such as the monoclonal antibody immobilization of granulocyte antigens (MAIGA) assay [57]. The GIFT is using donor granulocytes, making it necessary to

Table 2. Possible causes for neonatal neutropenia

Pregnancy-induced maternal hypertension/pre-eclampsia
Severe hemolytic disease of the fetus and newborn (HDFN)
Neonatal infection-induced neutropenia
Twin-twin transfusion
Congenital viral infections
Maternal drugs
Neonatal alloimmune neutropenia due to maternal alloantibodies (NAIN)
Neonatal neutropenia due to maternal autoantibodies
Autoimmune neutropenia of infancy

rule out that positive reactions are caused by anti-HLA-class-I antibodies or are due to nonspecific binding of circulating antibodies (immune complexes) via the Fc tail to the Fcγ receptors. In our laboratory, we test the patient sera before and after absorption of HLA-class-I antibodies using a pool of platelets. The GAT is known to have an enhanced ability to detect granulocyte agglutinins, such as anti-HNA-3a antibodies [57]. The MAIGA assay, a sandwich ELISA, can be used to detect HNA-1 (FcγRIIIb), pan-FcγRIIIb, HNA-2 (CD177), HNA-4 (CD11b), and/or HNA-5 (CD11a) antibodies. Until now, no CTL2(HNA-3)-specific monoclonal antibody is available for the MAIGA.

Antigen typing can be done with human antisera; however, nowadays genotyping is the standard procedure. For the different aspects concerning HNA genotyping, we refer to our recently published review [58] on the molecular basis and techniques currently available. Genotyping can be used to predict the HNA phenotype, except HNA-2 negativity. PCR with specific primers (PCR-SSP) is most frequently used; however, a cost-effective high throughput TaqMan assay for the genotyping of HNA-1, HNA-3, HNA-4, and HNA-5 has been developed. The more time-consuming sequence-based typing can be used for distinguish alleles encoding HNA-1a, HNA-1b, HNA-1c, or HNA-1d.

Treatment

Antibiotics for therapeutic treatment will often be sufficient [59]. Prophylactic treatment with antibiotics can be considered, especially in cases with severe neutropenia (<500/μl). However, evidence on effectiveness is lacking. Treatment with G-CSF is highly effective in increasing neutrophil numbers within days in a majority of cases and can be considered especially for neonates suffering from severe infections [60–62]. One should keep in mind that resistance to G-CSF in NAIN associated with anti-HNA-2 antibodies, possibly due to a G-CSF-induced increase of HNA-2 expression on the neutrophils, has been described [63–65]. Despite the stimulated production of neutrophils, controversial results on the benefit of G-CSF in terms of infection-free survival have been published [66–70].

Some case reports have been published in which benefits for the neonate have been achieved with second-line treatment with intravenous immunoglobulins (IVIg) [71]. However, there is not enough evidence to advice IVIg first-line treatment [72].

Conclusion

Even though NAIN is a relative rare condition, the consequences for the newborn can be serious, and timely diagnosis can prevent severe complications. A good differential diagnosis is important and can point the clinician in the right direction regarding the necessary investigation and treatment, and can predict the clinical course. This can also be reassuring in the counselling of the parents regarding the recovery of their child, and low hazards of severe disease in subsequent pregnancies. Laboratory investigation

for detection and specification of the causative maternal granulocyte-specific alloantibodies should be performed by experienced laboratories. In the majority of cases, therapeutic and prophylactic treatment with antibiotics is effective.

Disclosure Statement

Both authors have no conflict of interest.

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