A Neonatal Agranulocytosis

Hervé Delacour,1* Pierre Mornand,2 Sébastien Larréché,1 Jean Etienne Pilo,1 Audrey Mérens,1 and Patrick Imbert2

CASE DESCRIPTION

A 42-year-old gravida 5 para 3 woman delivered a male newborn at 36 weeks of gestation. She had no significant medical problems and her previous pregnancies led to healthy newborns. The current pregnancy was uneventful and the baby appeared healthy (body weight, 3190 g; Apgar score, 8/10/10). On the second day of life jaundice appeared. Laboratory tests revealed hyperbilirubinemia (total bilirubin 21.5 mg/dL; reference interval, <8.5 mg/dL), an agranulocytosis [absolute neutrophil count (ANC),3 <0.04 × 10⁹/L; reference interval, 5–21 × 10⁹/L], eosinophilia (0.83 × 10⁹/L; reference interval, <0.5 × 10⁹/L), and monocytosis (3.8 × 10⁹/L; reference interval, <1.1 × 10⁹/L). Hemoglobin (16.3 g/dL; reference interval, 14.5–22.5 g/dL), total white blood cell count (11.8 × 10⁹/L; reference interval, 9.4–34.0 × 10⁹/L), and platelet count (200 × 10⁹/L; reference interval, 150–300 × 10⁹/L) were within reference intervals. Microscopic examination of a May–Grünewald–Giemsa–stained blood smear confirmed agranulocytosis. There was no sign of infection. Additional testing included negative blood, gastric aspirate, and ear swab culture results and C-reactive protein concentration within the reference interval. Agranulocytosis was still present the following day (ANC, 0.064 × 10⁹/L). The jaundice was felt to be due to ABO hemolytic disease of the newborn (mother O negative, newborn B negative, direct antiglobulin test positive) and was treated successfully by a 36-h regimen of phototherapy. Conversely, agranulocytosis persisted (ANC, <0.04 × 10⁹/L on day 6).

1 Department of Biology and 2 Maternity and Pediatry Ward, Bégin Military Teaching Hospital, Saint Mandé, France.
* Address correspondence to this author at: Bégin Military Teaching Hospital, Department of Biology, 69 Avenue de Paris, 94 163, Saint Mandé Cedex, France. Fax +33-1-43-98-54-61; e-mail herve.delacour@santarm.fr.
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3 Nonstandard abbreviations: ANC, absolute neutrophil count; NAIN, neonatal alloimmune neutropenia; FNAIT, fetal and neonatal alloimmune thrombocytopenia; HDN, hemolytic disease of the newborn; HNA, human neutrophil antigen; GAT, granulocyte agglutination test; GIFT, granulocyte immunofluorescence test; MAIGA, monoclonal antibody-specific immobilization of granulocyte antigen; PCR-SSP, PCR with sequence-specific primers; HNA-1, HNA system 1.

QUESTIONS TO CONSIDER

1. What are the most common etiologies of neonatal neutropenia?
2. What additional testing should be performed in evaluating this patient?
3. What are the potential clinical consequences of neutropenia, particularly in the neonatal period?

DISCUSSION

The reference interval for ANC varies with age. Within the first 24 h of life, neutrophils constitute 60%–70% of the total white blood cell count and the lower limit of the reference interval is considered to be 5.0 × 10⁹/L. Because the total white blood cell count gradually declines after the first few days of life, the lower limit of the reference interval of the ANC is 1.5 × 10⁹/L during the first week and 1.0 × 10⁹/L from the second week to 6 months. After the first year, the ANC is normally >1.5 × 10⁹/L (1).

Neutropenia is frequently observed in neonates. It has been reported to occur in as many as 6% to 17% of all neonates admitted to intensive care units (2). The incidence of neutropenia is highest among preterm infants and rises with decreasing birth weight. In most cases, neutropenia is transient and confers no survival disadvantage. Some hematologic disorders and immunodeficiency diseases leading to neutropenia (e.g., Kostmann syndrome) may become apparent in the neonatal period, but these etiologies are rare. In the neonatal period, neutropenia suggests 4 common etiologies: (a) bacterial infection, (b) neonatal alloimmune neutropenia (NAIN), (c) neutropenia related to maternal hypertension, or (d) congenital viral infections (3).

The diagnostic approach is based on family and maternal history (neonatal neutropenia or unexplained death in siblings, maternal hypertension) and on clinical examination of both the mother and the newborn to identify any sign of infection. Examination of the mouth, rectum, and perineum is particularly important. Initial investigation should include cultures (blood, gastric aspirate, and ear swab), C-reactive protein and procalcitonin concen-
tration determinations, and microscopic examination of a blood smear. Additional tests could include viral testing (e.g., cytomegalovirus, Epstein-Barr virus, and parvovirus B19, as clinically indicated) and testing for granulocyte antibodies, which are pathognomonic of NAIN \(^3\).

NAIN is caused by feto-maternal granulocyte incompatibility. It is considered the granulocyte counterpart of fetal and neonatal alloimmune thrombocytopenia (FNAIT) and of hemolytic disease of the newborn (HDN). NAIN results from neutrophilic destruction by transplacentally acquired maternal neutrophil-specific IgG antibodies directed against paternally derived antigens. Although granulocytes express HLA class I at their surface, NAIN is mainly due to alloantibodies directed against neutrophil-specific antigens. Currently, the human neutrophil antigen (HNA) system comprises 7 antigens that are assigned to 5 glycoproteins (Table 1) \(^4\). Anti-HNA-1a and anti-HNA-1b have been the most commonly reported to cause NAIN. Although granulocytes express HLA class I at their surface, NAIN is mainly due to alloantibodies directed against neutrophil-specific antigens. Currently, the human neutrophil antigen (HNA) system comprises 7 antigens that are assigned to 5 glycoproteins (Table 1) \(^4\). Anti-HNA-1a and anti-HNA-1b have been the most commonly reported to cause NAIN. Anti-HNA-2a and anti-FcyRIIIb (also known as CD16) in mothers with an

<table>
<thead>
<tr>
<th>System</th>
<th>Antigens</th>
<th>CD</th>
<th>Gene</th>
<th>cDNA sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNA-1</td>
<td>HNA-1a</td>
<td>CD16</td>
<td>FCGR3B[^b]</td>
<td>NM_000570.4</td>
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<tr>
<td></td>
<td>HNA-1b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HNA-1c</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>HNA-1d</td>
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<td></td>
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</tr>
<tr>
<td>HNA-2</td>
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<td>CD177</td>
<td>NM_020406.2</td>
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<tr>
<td></td>
<td>HNA-2b</td>
<td></td>
<td></td>
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<tr>
<td>HNA-3</td>
<td>HNA-3a</td>
<td>CTL2[^c]</td>
<td>SLC44A2</td>
<td>NM_020428.3</td>
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<tr>
<td></td>
<td>HNA-3b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HNA-4</td>
<td>HNA-4a</td>
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<td></td>
<td>HNA-4b</td>
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<tr>
<td>HNA-5</td>
<td>HNA-5a</td>
<td>CD11b</td>
<td>ITGAL</td>
<td>NM_001114380.1</td>
</tr>
<tr>
<td></td>
<td>HNA-5b</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

\[^a\] Veldhuisen et al. (4).
\[^b\] Human genes: FCGR3B, Fc fragment of IgG, low affinity IIIb, receptor (CD16b); CD177, CD177 molecule; SLC44A2, solute carrier family 44 (choline transporter), member 2; ITGAM, integrin, alpha M (complement component 3 receptor 3 subunit); ITGAL, integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide).
\[^c\] CTL2, choline transporter-like protein 2.

### Table 2. Main features of the tests used for the detection of granulocyte antibodies.\(^a\)

<table>
<thead>
<tr>
<th>Test</th>
<th>Principles</th>
<th>Main Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAT</td>
<td>Serum from the patient is incubated with a granulocyte suspension. The reaction is graded from negative to 4+ on the basis of the percentage of cells that are agglutinated.</td>
<td>Possible false-positive results due to spontaneous agglutination of neutrophils.</td>
</tr>
<tr>
<td>Indirect GIFT</td>
<td>Serum from the patient is incubated with a granulocyte suspension. After a wash step, bound antibodies to granulocytes are detected using a fluoresceinated human antiimmunoglobulin.</td>
<td>Possible false-positive results due to immune complexes.</td>
</tr>
<tr>
<td>MAIGA</td>
<td>Granulocytes are incubated with the serum from the patient. This granulocyte suspension is washed and incubated with a murine monoclonal antibody to a specific neutrophil glycoprotein. After another wash step, the granulocyte membranes are disrupted. The resulting lysate is then transferred to microwells coated with antimouse immunoglobulins. Detection of the trimolecular neutrophil antigen–patient HNA antibody–murine monoclonal antibody complex is detected by the addition of antihuman IgG conjugated to horseradish peroxidase followed by a substrate.</td>
<td>Very complex test requiring highly skilled staff.</td>
</tr>
</tbody>
</table>

\[^a\] Clay et al. (6).
HNA-null phenotype [i.e., FcγRIIIb deficiency (Box 1)] are more rarely involved (5).

Demonstration of granulocyte antibodies must be performed by using a minimum of 2 methods, the granulocyte agglutination test (GAT), the indirect granulocyte immunofluorescence test (GIFT), or the monoclonal antibody-specific immobilization of granulocyte antigen (MAIGA) assay (Table 2). Antibody identification is verified by determination of maternal, paternal, and neonatal human neutrophil antigens (HNA). The mother will be antigen negative to the alloantibody present in maternal/neonatal serum. The father and neonate will be antigen positive. PCR with sequence-specific primers (PCR-SSP) is most widely used for HNA genotyping (7).

In this case, IgG antigranulocyte antibodies were detected at a high level in maternal serum using GIFT but were negative with GAT even after serum dilution (from 1:2 to 1:512). A high concentration of anti-CD16 was identified by MAIGA, with maternal neutrophils being typed as CD16 negative. HNA system 1 (HNA-1) genotyping was performed by PCR-SSP. The mother was negative for HNA-1a, -1b, and -1c, consistent with an FcγRIIIb deficiency, whereas the newborn and the father were positive for HNA-1a and -1b and negative for -1c. NAIN due to anti-FcγRIIIb antibodies was diagnosed.

NAIN is an uncommon disorder, with an estimated incidence 1 in 2000 live births. This is probably an underestimate, however, because NAIN is often clinically silent, as in the current case. A prospective study, performed in an intensive care unit, showed that NAIN causes 2.9% of neonatal neutropenia (2).

The exact mechanism underlying maternal immunization remains unknown. The rate of alloimmunization is variable and depends on the frequency of the antigens in the population and on their immunogenicity. However, as for RHD and FNAIT, NAIN incidence is lower than alloimmunization incidence, which is itself lower than the feto-maternal incompatibility.

Like FNAIT, and contrary to HDN due to Rh (and most other red cell antigens except ABO), NAIN can occur during a first pregnancy. There is no risk to the baby during pregnancy, and the course of pregnancy is uneventful. The neonate is usually asymptomatic, with neutropenia often being found incidentally. Sometimes the neonate may develop a mild infection (e.g., omphalitis and other skin infections, otitis, or urinary infections), and severe cases have been reported (e.g., pneumonia, sepsis, or meningitis). However, as neutropenia may also be an early presenting sign of sepsis in neonates, it can be difficult to determine whether the sepsis preceded the neutropenia or vice versa. In NAIN, neutropenia is typically associated with monocytosis and sometimes with eosinophilia. Hemoglobin values and platelet counts are within the reference intervals, as in this case (3). Bone marrow aspiration, which is rarely performed, typically shows a normocellular marrow with late granulocytic maturation arrest.

Neutropenia may last up to 6 months (median, 7 weeks). Serial blood cell counts should be performed until correction of neutropenia, which is usually preceded by a decrease in monocytes. NAIN management is mainly based on prevention of infection. Skin hygiene and careful disinfection of baby bottles and nipples are essential. Prophylactic antibiotic therapy, intravenous immunoglobulins, and recombinant human granulocyte colony–stimulating factor therapy remain controversial. A rapid discharge from the hospital, even in the absence of correction of neutropenia, is recommended by many authors to limit potential exposure to hospital-acquired infections. This approach should be considered only if the parents are able to quickly detect signs of infection and refer the neonate to the hospital (3).

The risk of NAIN recurrence is very high in subsequent pregnancies and depends on whether the father’s granulocyte genotype is homozygous or heterozygous for the targeted antigen. Although no specific investigation is required during pregnancy, a blood cell count should be performed at birth and appropriate clinical management instituted in case of neutropenia.

In this case, no infectious complication was observed and, without any specific treatment, ANC spontaneously increased to 7.1 × 10^9/L on day 21.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 re-
quirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References


Box 1

FcγRIIIb: Functions and Consequences of Its Deficiency (8–9).

Human FcγRIIIb (FcγRIIIb, CD16) is a low-affinity Fc receptor for IgG1 and IgG3, constitutively expressed with a high density (100 000–400 000 copies per cell) only by cells of granulocytic lineage. It binds with its membrane proximal domain to the Fc region of polymeric IgG antibodies. Resting neutrophils primarily engage FcγRIIIb for the binding of immune complexes and clear them from the circulation. The receptor also contributes to phagocytosis of opsonized microorganisms. The HNA-1–null phenotype is more frequent in Sub-Saharan Africans (approximately 4%) than in whites (0.2%–0.8%) and nearly absent in Asians and Amerindians. Paradoxically, as in the mother in this case, most FcγRIIIb-deficient individuals do not suffer from repeated infections or autoimmune or immune-complex diseases, although this receptor has a key role in immune defense. Further investigations are required to explain this phenomenon.

Commentary

Lori Luchtman-Jones*

Best practices combine clinical acumen and selected testing for diagnosis and management. I nominate neonatal neutropenia as a diagnosis in need of best practices guidelines. As clinicians striving to provide excellent care and stretch those healthcare dollars, neonatologists face additional diagnostic challenges in tiny patients with limited blood volume and vascular access.

Neutropenia is common in neonates, particularly during the first week of life, in premature or sick in-

fants, and in babies receiving multiple medications. Fortunately, neutropenia is often (but not always) both transient and unassociated with severe morbidity or mortality. Serious conditions include bone marrow failure syndromes, acute leukemia, and bone marrow infiltration. With a benign maternal history and postnatal course, other blood cell abnormalities, dysmorphic features, severe or recurrent infections, eczema, diarrhea, or persistent neutropenia beyond 2 to 3 months might trigger further testing. Severe infection risk increases with duration and severity of neutropenia, with neutrophil dysfunction, and with other immune alterations. A critical early consideration is whether lymphopenia with a severe immunodeficiency syndrome is present.

Because expectant management is the mainstay for the asymptomatic neutropenic neonate, the di-

George Washington University Medical School and Center for Cancer and Blood Disorders, Children’s National Medical Center, Washington, DC.

* Address correspondence to the author at: Children’s National Medical Center, 111 Michigan Ave., N.W., Washington, DC 20010. E-mail: lljones@childrensnational.org.

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