

Neonatal Lupus or Not? An Intriguing Immunological Dilemma

Silvia Beretta, MD, Valentina Fabiano, MD, and Gian Vincenzo Zuccotti, MD

Anti-Ro antibodies appear to represent a direct pathogenic factor in neonatal lupus (NL) neutropenia. Resolution of NL neutropenia is expected when anti-Ro antibodies are cleared. In this report, we describe a case of persistent neutropenia in a baby born to an anti-Ro-positive mother. The initial diagnosis of NL neutropenia was later called into question due to the identification of positive anti-neutrophil antibodies and transient hypogammaglobulinemia of infancy. The patient seemed to have had overlapping causes of her hematologic manifestations. To our knowledge, no other similar cases have been previously reported in the literature. Newborns are not routinely tested for white blood cell count, and several neutropenia cases remain undetected.

Background

NEONATAL LUPUS (NL) is an uncommon passively acquired autoimmune disease that results from the transplacental passage of the autoantibodies from mother to fetus,^{1,2} with maternal immunoglobulin G (IgG) being actively transported across the placenta to the fetus at about 16 weeks of gestation. The NL diagnosis is generally clinically based on the presence of maternal and neonatal autoantibodies. Only about 1%–2% of the affected infants' mothers are symptomatic.³ The NL symptoms are various and variable, involving the heart, skin, liver, and blood. The most serious clinical manifestation is a complete heart block,^{4,5} and liver involvement may also cause liver failure and cholestasis.⁶ A skin rash is usually self-limiting and transient.² The hematologic involvement may cause transient anemia, neutropenia, and thrombocytopenia. About 21% of NL-affected newborns show transient neutropenia, and in 56% of cases, neutropenia is isolated.⁷ Aspecific echoencephalographic alterations have also been described.^{8,9}

Case Presentation

MP is a female baby born at the 39th week of pregnancy, in October 2011, by cesarean section because of failed labor induction, without complications. The Apgar score was 9 at the first minute and 10 at the fifth minute after birth. The baby was healthy and her body weight was 3,115 g, adequate for her gestational age (25th–50th percentile). Her mother, who was affected by Sjögren's syndrome, presented positive for antinuclear antibodies (ANA), anti-Ro (SSA), and anti-La (SSB). The mother did not present any hematologic disturbances and did not follow any specific treatment

for Sjögren's syndrome during pregnancy, but because of signs of premature labor, on August 2011, 2 doses of betamethasone were administered to induce fetal lung maturation. She was also treated with a tocolytic drug and penicillin. Complete blood examinations, already scheduled for the baby's first day of life because of the maternal autoimmune disease, were performed earlier (a few hours after birth) because of the presence of an ecchymotic skin lesion and demonstrated pancytopenia [white blood cells (WBC): 4,700/mm³; neutrophils 1,120/mm³; hemoglobin 12.7 g/dL; and platelets 82,000/mm³]. The coagulation profile was determined to be normal. Hemolysis, as determined by a direct Coombs test, was negative and infectious causes were also excluded. Autoantibody testing, performed on the same day, showed ANA 1:640 positive (speckled pattern), anti-Ro >240 U/mL, and anti-La negative. An electrocardiogram, performed on the third day of life, showed a normal sinus rhythm without any sign of heart block. Cerebral and abdominal ultrasounds were also performed and were normal. On the fifth day of life, WBC and platelet counts were normalized, while a severe neutropenia (370/mm³) still persisted. Based on maternal history, autoantibody positivity, and the presence of neutropenia, we diagnosed the baby an NL. On her sixth day of life, the baby was discharged and strictly followed up. She showed an optimal growth and did not present any relevant health problem even though she continued to present a persistent mild to moderate neutropenia at 1, 3, 6, 9, and 12 months of age (Table 1). Complicating matters, at 3 months of life, the baby's IgA and IgM were low (IgA 0.05 g/L; IgM 0.13 g/L), and IgG levels were at the lower limit of the normal range (IgG 3.73 g/L), and at 6 months, Ig A-M-G deficits (IgA 0.04 g/L; IgM 0.24 g/L IgG 2.14 g/L) were present¹⁰ (Table 2), without CD4/CD8

TABLE 1. TREND OF BABY'S NEUTROPHIL COUNT IN THE FIRST 12 MONTHS

Age (months)	Neutrophil count (n/mm ³)	Normal neutrophil values for age (n/mm ³) [range 95% CI (mean)] ¹¹
1	760	1,000–9,000 (3,800)
3	530	N/A
6	950	1,000–8,500 (3,800)
9	740	N/A
12	990	1,500–8,500 (3,500)

Normal values for age as available.¹¹

N/A, not available.

abnormalities, with a normal lymphocyte B count, and with normal serum levels of specific antibodies to hepatitis B virus and tetanus toxoid vaccines after administration of 2 doses of the vaccines as scheduled in the Italian pediatric vaccination plan. Genetic tests to identify common variable immunodeficiencies [mutations of inducible T-cell costimulator, transmembrane activator and calcium modulator and cyclophilin ligand interactor, B-cell activating factor-receptor, B-cell receptor complex (CD19, CD21, and CD81), and CD20] were performed and were negative. Therefore, we hypothesized that she had a transient hypogammaglobulinemia of infancy. During her clinical follow-up, MP did not experience any serious or recurrent infection, and she showed appropriate somatic and neurologic development. At 12 months of life, ANA testing was negative, anti-Ro antibodies remained slightly positive [16 U/mL (<7=neg)], which was possibly consistent with a mildly persistent maternal anti-Ro due to a reduced Ig catabolism, a mild neutropenia still persisted (990/mm³), IgG and IgM levels were normal (IgG 4.09 g/L and IgM 0.36 g/L), and IgA level was still low (0.02 g/L; Table 2), indicating a selective IgA deficiency. Due to the persistence of neutropenia at 1 year of age, we called into question our diagnosis of NL since, if we were right, we would have expected a resolution of the neutropenia in about 8–10 months. To consider possible differential diagnosis, at 12 months of age, we tested the baby and her mother for autoimmune neutropenia (AIN) and found anti-neutrophil antibodies only in the baby's serum. The method used for evaluating the presence of anti-neutrophil antibodies was the indirect granulocyte immunofluorescence test (GIFT), and a positive reaction was found for 3 of the 4 cellular suspensions analyzed (suspension 1=0.5; suspension 2=1.5; suspension 3=2.9; suspension 4=2.8; expressed as fluorescence displacement, [<1.4=neg]). We continued to follow up the baby, who always remained healthy and continued to show normal growth. She performed all the other planned vaccines without any problem. At 14 months of age, a second evaluation for

the presence of GIFT showed a persisting positive reaction for only 2 of the 4 cellular suspensions analyzed (suspension 1=1.1; suspension 2=4.5; suspension 3=1.1; suspension 4=2.6; expressed as fluorescence displacement, [<1.4=neg]). At 18 months of age, her neutrophil counts showed an increase, reaching 1,500/mm³, which is considered normal for her age.¹¹

Conclusions

Autoantibodies to the Ro/La ribonucleoprotein particles are sometimes found in the sera of systemic lupus erythematosus (SLE) and Sjögren's syndrome affected patients. Moreover, patients affected by SLE and Sjögren's syndrome, who are positive for anti-Ro antibodies, have lower WBC counts than those without anti-Ro.¹² Anti-Ro antibodies seem to be a direct pathogenic factor in NL neutropenia. Kanagasagar et al. identified anti-Ro as a direct participant in the pathogenic processes causing neutropenia,⁷ as they bind the cell surface of neutrophils, leading to complement fixation.¹³ Neutropenia in NL is usually transient and mild and does not require any treatment. The baby should be periodically followed up until neutropenia is resolved. In a case report of severe neutropenia in an NL patient, rh-G-CSF has been successfully administered. The authors speculated that pathogenesis of neutropenia may also be explained by hyposensibility of the medullary cell receptors to the growth factor.¹⁴

Transient hypogammaglobulinemia of infancy is a temporary disease of unknown cause. Various hypotheses of pathogenetic mechanisms have been postulated, such as a defect in the T-helper cell maturation,¹⁵ the presence of alloantibodies crossing the placenta and causing transient suppression of fetal Ig production,¹⁶ the genetic heterozygosity for different immunodeficiency diseases, which some hypothesized based on the findings of transient hypogammaglobulinemia in relatives of patients affected by immunodeficiency disorders.¹⁷ The diagnostic criteria are not standardized. Several patients are asymptomatic, while others are affected by recurrent infections.^{18,19} The immunization responses are normal.²⁰ As the child ages, the number and condition of T-helper cells improve and this situation corrects itself. Associated hematologic manifestations, such as transient neutropenia, are infrequently described.¹⁸

AIN is caused by granulocyte-specific antibodies and has been associated with a variety of underlying diseases, including infections, collagen vascular diseases, primary abnormalities of B or T lymphocytes or natural killer cells, a deficiency of regulatory T cells, immune thrombocytopenic purpura, and autoimmune hemolytic anemia.^{21,22} In most cases, however, AIN is not associated with other illnesses or underlying disease, and is referred to as chronic benign neutropenia. In pediatrics, this syndrome typically occurs in infants between the ages of 5 and 15 months, but the range extends from 1 month to adulthood. AIN is typically characterized by spontaneous remission with the disappearance of autoantibodies.²³ Specific treatment of neutropenia is not usually required. Many patients remain free from infections and require no or minimal medical intervention. Observation of serial blood counts is the most common practice. Only in the presence of serious infections is a therapy with corticosteroids or G-CSF considered for increasing neutrophil counts.¹¹

TABLE 2. TREND OF BABY'S ANTI-RO AND IGA-M-G LEVELS IN THE FIRST 12 MONTHS

Age (months)	Anti-Ro (U/mL)	IgA-M-G (g/L)
1	>240	N/A
3	N/A	0.05–0.13–3.73
6	N/A	0.04–0.24–2.14
9	N/A	N/A
12	16	0.02–0.36–4.09

N/A, not available.

AIN must be distinguished from more serious forms, such as neutropenia associated with immunodeficiencies (hyper- or hypogammaglobulinemia, T-cell defects, or natural killer dysfunctions), which lead to recurrent and severe infections.

In a previously reported case of persistent neutropenia in NL, anti-neutrophil antibodies were negative. Neutropenia persistence was explained by the possible existence of autoantibodies or T lymphocytes that bind to and injure progenitor cells in the bone marrow.¹³ In another case, the severity of the baby's neutropenia was attributed to the high titer of anti-Ro or to the high cross-reactivity of autoantibodies with the antigen of the mother's and newborn's neutrophils. In this case, both mother and baby presented neutropenia.¹⁴ In our case, the mother's anti-Ro titer was also high, but she did not show any hematologic alterations. Moreover, in our case, both anti-Ro and anti-neutrophil antibodies were positive, making the clear understanding of the underlying pathogenetic mechanisms causing neutropenia more difficult. Our patient seemed to have overlapping causes of her hematologic manifestations. At this point of baby's history, after observing a spontaneous resolution of neutropenia, it is hard to determine which pathogenetic mechanism, either autoimmunity or a passive antibody transplacental passage, weighted more on baby's neutropenia. Based on the accuracy of our initial diagnosis of NL, we did not perform anti-neutrophil antibody testing at birth or in the baby's first days of life, so we could not compare the positive titers that we found at 14 months of age to a basal value. This is a limitation of the present case. The severity of baby's neutropenia in her first months of life may have been the result of both mechanisms acting synergistically. The benign clinical course of our case did not suggest a form of neutropenia associated with immunodeficiencies. In our case, transient hypogammaglobulinemia and neutropenia were more probably 2 distinct findings rather than associated manifestations of the same disorder.

To our knowledge, no other similar cases have been previously reported in the literature. Nevertheless, unless ill, newborns and babies are not routinely tested for WBC counts, so several neutropenia cases remain undetected, suggesting that this hematologic alteration in infancy may be more frequent than expected.

Authors' Contributions

S.B. participated in acquisition, analysis, and interpretation of data, and drafted the article.

V.F. participated in acquisition, analysis, and interpretation of data, and drafted the article.

G.V.Z. participated in acquisition, analysis, and interpretation of data, and revised and gave final approval of the article.

Author Disclosure Statement

The authors declare no conflict of interests.

References

1. Brucato A, Buyon JP, Horsfall AC, Lee LA, Reichlin M. Fourth international workshop on neonatal lupus syndromes and the ro/ssa-la/ssb system. *Clin Exp Rheumatol* 1999; 17:130–136.
2. Lee LA. Neonatal lupus: clinical features, therapy, and pathogenesis. *Curr Rheumatol Rep* 2001; 3:391–395.
3. Brucato A, Cimaz R, Caporali R, Ramoni V, Buyon J. Pregnancy outcomes in patients with autoimmune diseases and antiRo/SSA antibodies. *Clin Rev Allergy Immunol* 2011; 40:27.
4. Buyon JP, Hiebert R, Copel J, Craft J, Friedman D, Katholi M, Lee LA, Provost TT, Reichlin M, Rider L, Rupel A, Saleeb S, Weston WL, Skovron ML. Autoimmune-associated congenital heart block: demographics, mortality, morbidity and recurrence rates obtained from a national neonatal lupus registry. *J Am Coll Cardiol* 1998; 31:1658–1666.
5. Alexander E, Buyon JP, Provost TT, Guarnieri T. Anti-Ro/SS-A antibodies in the pathophysiology of congenital heart block in neonatal lupus syndrome, an experimental model. *In vitro* electrophysiologic and immunocytochemical studies. *Arthritis Rheum* 1992; 35:176.
6. Lee LA, Reichlin M, Ruyle SZ, Weston WL. Neonatal lupus liver disease. *Lupus* 1993; 2:333–338.
7. Kanagasagar S, Cimaz R, Kurien BT, Brucato A, Scofield RH. Neonatal lupus manifests as isolated neutropenia and mildly abnormal liver functions. *J Rheumatol* 2002; 29:187–191.
8. Silverman E, Jaeggi E. Non-cardiac manifestations of neonatal lupus erythematosus. *Scand J Immunol* 2010; 72:223.
9. Zuppa AA, Fracchiolla A, Cota F, Gallini F, Savarese I, D'Andrea V, Luciano R, Romagnoli C. Infants born to mothers with anti-SSA/Ro autoantibodies: neonatal outcome and follow-up. *Clin Pediatr* 2008; 47:231–236.
10. Asku G, Genel F, Koturoglu G, Kurugöl Z, Kütükçler N. Serum immunoglobulin (IgG, IgM, IgA) and IgG subclass concentrations in healthy children: a study using nephelometric technique. *Turk J Pediatr* 2006; 48:19–24.
11. Segel GB, Halterman JS. Neutropenia in pediatric practice. *Pediatr Rev* 2008; 29:12–13.
12. Alexander EL, Arnett FC, Provost TT, Stevens MB. Sjögren's syndrome: association of anti-Ro(SSA) antibodies with vasculitis, hematologic abnormalities, and serologic hyperreactivity. *Ann Intern Med* 1983; 98:55–59.
13. Kurien BT, Newland J, Paczkowski C, Moore KL, Scofield RH. Association of neutropenia in systemic lupus erythematosus with antiRo and binding of an immunologically crossreactive neutrophil membrane antigen. *Clin Exp Immunol* 2000; 120:209–217.
14. Zuppa AA, De Luca D, De Turre P, Cota F, Tortorolo G. Usefulness of rh-G-CSF in early-onset severe neutropenia in neonatal lupus syndrome. *J Pediatr Hematol Oncol* 2004; 26:609–611.
15. Siegel RL, Issekutz T, Schwaber J, Rosen F, Geha RS. Deficiency of T-helper cells in transient hypogammaglobulinemia of infancy. *NEJM* 1981; 305:1037.
16. Fudenberg HH. Antibody to hereditary human gamma-globulin (GM) factor resulting from maternal-fetal incompatibility. *Science* 1964; 145:170–171.
17. Soothill JF. Immunoglobulins in first-degree relatives of patients with hypogammaglobulinemia. Transient hypogammaglobulinemia: a possible manifestation of heterozygosity. *Lancet* 1968; 1:1001.
18. Dalal I, Reid B, Nisbet-Brown E, Roifman CM. The outcome of patients with hypogammaglobulinemia in infancy and early childhood. *J Pediatr* 1998; 133:144.
19. Qian JH, Zhu JX, Zhu XD, Chen TX. Clinical features and follow-up of Chinese patients with symptomatic

- hypogammaglobulinemia in infancy. *Chin Med J (Engl)* 2009; 122:1877.
20. Kiliç SS, Tezcan I, Sanal O, Metin A, Ersoy F. Transient hypogammaglobulinemia of infancy: clinical and immunologic features of 40 new cases. *Pediatr Int* 2000; 42:647–650.
 21. Lima CS, Paula EV, Takahashi T, Saad ST, Lorand-Metze I, Costa FF. Causes of incidental neutropenia in adulthood. *Ann Hematol* 2006; 85:705–709.
 22. Nakamura K, Miki M, Mizoguchi Y, Karakawa S, Sato T, Kobayashi M. Deficiency of regulatory T cells in children with autoimmune neutropenia. *Br J Haematol* 2009; 145:642–647.
 23. Bux J, Behrens G, Jaeger G, Welte K. Diagnosis and clinical course of autoimmune neutropenia in infancy: analysis of 240 cases. *Blood* 1998; 91:181–186.

Address correspondence to:
Silvia Beretta, MD
University of Milan
Department of Pediatrics
Luigi Sacco Hospital, Milan
Via GB Grassi 74
Milan 20157
Italy

E-mail: beretta.silvia@hsacco.it

Received for publication March 16, 2014; accepted after revision April 28, 2014.