

A late preterm infant with lymphopenia

Shazia Lutfeali, M.D., David A. Khan, M.D., and Christian Wysocki, M.D.

ABSTRACT

The newborn screen for severe combined immunodeficiency (SCID) uses real-time quantitative polymerase chain reaction for T-cell receptor excision circles and is highly sensitive for SCID. However, T-cell lymphopenia from other primary and secondary causes, such as DiGeorge syndrome, prematurity, thymic involution from stress, and thymectomy during cardiac surgery, is also detected. We present a newborn girl with T-cell lymphopenia of unknown etiology detected via abnormal newborn screen.

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CHIEF CONCERN

Abnormal newborn screen.

HISTORY OF PRESENT ILLNESS

The patient was born at 34 weeks by cesarean section for fetal decelerations. The pregnancy reportedly was uncomplicated. Maternal antenatal betamethasone was administered for lung development, and the postnatal course was unremarkable. Birth weight was 2325 g. Newborn screens obtained at 1 and 10 days of life resulted with very low T-cell receptor excision circles (TREC). The patient was referred to the immunology clinic. Her pediatrician was contacted immediately, and bloodwork was sent. On evaluation, the patient was formula-feeding well and had not developed infections, rash, or diarrhea. The family history was negative for primary immunodeficiency, consanguinity, and unexplained deaths.

PHYSICAL EXAMINATION

The patient appeared well, with no thrush, midline defects, pathologic heart murmurs, abnormal facial features, hypotonia, or other abnormalities. Her weight was appropriate for age. Flow cytometry at 2 weeks of age showed severe pan-lymphopenia, with an absolute lymphocyte count of 551 cells/ μ L, CD3⁺ 429 cells/ μ L, CD3⁺ CD4⁺ 342 cells/ μ L, CD3⁺ CD8⁺ 68 cells/ μ L,

CD3⁺ CD19⁺ 2 cells/ μ L, and CD3⁺ CD16/56⁺ 104 cells/ μ L. Naive T-cell percentages were normal, with 73% naive CD4⁺ cells and 93% naive CD8⁺ cells. No other hematologic abnormalities were detected. Proliferation to phytohemagglutinin was low-normal.

QUESTIONS

Question 1: What is the Differential Diagnosis?

Primary causes of low TRECs include classic and “leaky” severe combined immunodeficiency (SCID), Omenn syndrome, complete or partial DiGeorge syndrome, CHARGE (coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities) syndrome, and ataxia telangiectasia. Secondary causes include prematurity, surgical thymectomy, neonatal leukemia, gastroschisis, and human immunodeficiency virus. Although the patient did not meet diagnostic criteria for classic SCID, there was concern for leaky SCID. Given T-, B-, and natural killer (NK)-cell lymphopenia, adenosine deaminase (ADA), and purine nucleotide phosphorylase (PNP) deficiencies were considered.

Question 2: What Additional Laboratory Data or Investigations Would Be Helpful in Arriving at a Diagnosis in This Patient?

ADA and PNP enzymatic activity can be assessed with rapid turnaround. Genetic analysis for combined immunodeficiencies and 22q11.2 deletion should be strongly considered. Human immunodeficiency virus testing is also warranted.

ADDITIONAL MEDICAL HISTORY

The mother revealed that she had undergone a kidney transplantation for lupus nephritis 3 years earlier

From the Division of Allergy and Immunology, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas

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Address correspondence to Shazia Lutfeali, M.D., Division of Allergy and Immunology, Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, F4.206, Dallas, TX 75390-8859

E-mail address: shazia.lutfeali@gmail.com

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Table 1 Trend of patient's immunologic studies

Immunologic Study	Reference Range	Age		
		2 Wk	1 Mo	4 Mo
Lymphocytes, cells/ μ L				
Absolute lymphocyte count	3400–7600	551	1979	7107
CD3 ⁺	2500–5500	429	776	3895
CD3 ⁺ CD4 ⁺	1600–4000	342 (73% naive)	587 (86% naive)	3042 (89% naive)
CD3 ⁺ CD8 ⁺	560–1700	68 (93% naive)	166 (94% naive)	832 (96% naive)
CD3 [−] CD19 ⁺	300–2000	2	975	2497
CD3 [−] CD16/56 ⁺	170–1100	104	170	571
Immunoglobulin, mg/dL				
A	7.0–48.0	<7.8	—	<7.8
G	310.0–650.0	570	—	554
M	4.0–60.0	<5.3	—	39.3
E	0.0–12.0	21.5	—	4.4

and had taken hydroxychloroquine, tacrolimus, azathioprine, and prednisone throughout her pregnancy.

ADDITIONAL LABORATORY STUDIES

TRECs rechecked at 2 weeks of age had normalized at 8852 per 1,000,000 CD3⁺ T cells (reference value >6794). Flow cytometry repeated at 4 weeks showed absolute lymphocytes of 1980 cells/ μ L, CD3⁺ 776 cells/ μ L, CD3⁺ CD4⁺ 587 cells/ μ L (86% naive), CD3⁺ CD8⁺ 166 cells/ μ L (94% naive), CD3[−] CD19⁺ 975 cells/ μ L, and CD3[−] CD16/56⁺ 170 cells/ μ L (Table 1). ADA and PNP enzymatic activities were normal.

CLINICAL COURSE

As TRECs normalized, proliferation to phytohemagglutinin was normal for age, and B- and T-cell lymphopenia improved by 1 month of age, a watchful waiting approach was adopted. Trimethoprim-sulfamethoxazole and other antimicrobial prophylaxis were held. The mother did not wish to breast-feed; she was advised to avoid public places and people with known illnesses, and to boil water for infant formula. Live viral vaccines were held. All lymphocyte subsets normalized by age 4 months and immunoglobulin G (likely reflective of maternal levels) remained stable at 554 mg/dL. At age 10 months, the patient had protective titers to tetanus, diphtheria, *Haemophilus influenzae* B, and 7 of 13 Prevnar (Wyeth Pharmaceuticals Inc., Philadelphia, PA) vaccine serotypes. She was cleared for live viral vaccines, which she tolerated well.

DISCUSSION

Previous studies showed that maternal immunosuppressive medications during pregnancy can cause transient neonatal lymphopenia and abnormal newborn screening test results for SCID. Kuo *et al.*¹ published information about two infants with low TRECs and

severe T-cell lymphopenia, with prenatal exposure to azathioprine and mercaptopurine. A predominance of naive T cells, normal proliferation to mitogens, and the presence of thymic shadow on a chest radiograph were reassuring.¹ Both infants received infectious prophylaxis and/or supplemental immunoglobulin, and T-cell counts normalized by age 5 to 6 months.¹ Singla *et al.*² reported an infant with prenatal exposure to azathioprine who presented with abnormal TRECs, severe lymphopenia, and decreased naive T-cell percentages. Primary immunodeficiency genetic testing was negative, and lymphocyte counts spontaneously improved.²

Thomas *et al.*³ reported about an infant with undetectable TRECs and with B-, T-, and NK-cell lymphopenia, whose mother was on azathioprine. The infant received infectious prophylaxis, and laboratory abnormal results improved by 3 months.³ Conversely, a study by de Felipe *et al.*⁴ described two full-term neonates with normal TRECs but low kappa-deleting recombination excision circles (KRECs), which suggests B-cell lymphopenia. Abnormal values were attributed to maternal azathioprine during pregnancy. KRECs in one infant remained low at 1 month but normalized after breast-feeding cessation.⁴ Another 2-year study, by Barbaro *et al.*,⁵ identified 13 infants with transiently low KRECs whose mothers were on azathioprine, mercaptopurine, and/or tacrolimus during pregnancy. Again, values normalized without intervention.⁵ These studies demonstrate that use of maternal immunosuppressive medications during pregnancy can affect both B- and T-cell lines in the neonate (Table 2).

Azathioprine causes immunosuppression partially by interfering with nucleic acid synthesis in rapidly dividing cells, including lymphocytes. Although there may be associations with prematurity and low birth weight, azathioprine is considered relatively safe during pregnancy.⁶ *In vitro* studies showed increased sensitivity of B cells over T cells to drug-induced apoptosis,⁷

Table 2 Summary of cases that attributed low TRECs or KRECs in patients to maternal immunosuppressive medications

Study	Laboratory Test Result Abnormalities	Medication	Interventions	Time to Marked Improvement or Normalization of Flow Cytometry
Kuo <i>et al.</i> ¹	↓ TREC	Azathioprine (<i>n</i> = 1), mercaptopurine (<i>n</i> = 1)	Infectious prophylaxis and/or supplemental immunoglobulin	5–6 mo
Singla <i>et al.</i> ²	↓ TREC	Azathioprine (<i>n</i> = 1)	None	3 wk
Thomas <i>et al.</i> ³	↓ TREC	Azathioprine (<i>n</i> = 1)	Infectious prophylaxis	3 mo
de Felipe <i>et al.</i> ⁴	↓ KREC	Azathioprine (<i>n</i> = 1)	None	After breast-feeding cessation
Barbaro <i>et al.</i> ⁵	↓ KREC	Azathioprine (<i>n</i> = 9), mercaptopurine (<i>n</i> = 1), azathioprine and tacrolimus (<i>n</i> = 3)	None	2–10 wk

↓ = low; TREC = T-cell receptor excision circles; KREC = kappa-deleting recombination excision circles.

which may explain why KRECs were preferentially affected in the European studies described.^{4,5} A dose-dependent effect of maternal azathioprine on neonatal lymphopenia may also influence which, if any, neonatal cell lines are affected.⁸

Final Diagnosis

Transient neonatal lymphopenia from maternal immunosuppressive medications.

CONCLUSION

We presented an infant girl with very low TRECs, found to have severe lymphopenia, who spontaneously recovered by age 4 months. This case highlighted the importance of a thorough medical history because the mother's condition was not divulged without probing. Lymphopenia was probably multifactorial, from maternal immunosuppressive medications (particularly azathioprine), antenatal steroids, perinatal stress, and prematurity. Maternal immunosuppression, therefore, should be considered in the differential diagnosis of a neonate with lymphopenia. In general, the long-term effects of maternal immunosuppressive medications on neonatal immune function are not well established, but spontaneous normalization in all of the above cases suggested that the effects were transient.

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