

Ruling out HIV infection when testing for severe combined immunodeficiency and other T-cell deficiencies

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Activity Objectives

1. To distinguish the features of congenital infections with associated immune suppression from primary immune deficiency states, specifically severe combined immune deficiency (SCID).
2. To apply knowledge of the immunoassays available and their interpretation to confirm or exclude primary immune deficiency.
3. To identify the appropriate assays for diagnosis or exclusion of perinatal HIV infection in infancy.

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Key words: *Diagnosis of severe combined immunodeficiency versus pediatric HIV infection, nonantibody test for HIV in children less than 18 months of age, T-cell receptor excision circle assay, T-cell lymphocytopenia in patients with severe combined immunodeficiency, genetic sequencing for types of severe combined immunodeficiency*

CLINICAL VIGNETTE

A 7-month-old male infant was transferred to a tertiary care children's hospital for evaluation of cough, fever,

hypoalbuminemia, and failure to thrive. The infant's neonatal course was unremarkable, and he received routine childhood immunizations at 2 and 4 months of age. His onset of symptoms began at 2 months of age with persistent otitis media, cough, and diarrhea. His cough progressed to respiratory failure, necessitating mechanical ventilation for 1 week. No pathogen was isolated. After extubation, he acquired *Shigella* species bacteremia during his hospitalization. His discharge was short-lived, and he was again admitted to the hospital with pneumonia, persistent loose stools, and peripheral edema with hypoalbuminemia. At the time of transfer to the hospital, he was febrile (38.9°C) with a respiratory rate of 30 breaths/min and heart rate of 100 beats/min. His chest radiograph demonstrated hazy infiltrates across all lung fields but with upper lobe predominance (see Fig E1 in this article's Online Repository at www.jacionline.org). There was fine diffuse nodularity throughout all lung fields without evidence of bronchiectasis or cystic changes. The nodularity was consistent with interstitial atypical infection, possibly caused by *Pneumocystis jiroveci* or a viral process, such as nodular hyperplasia. The mediastinum and hilum were reviewed by using computed tomography and showed no evidence of adenopathy. The heart and bony structures were without abnormality. Results of the physical examination were remarkable for oral thrush, bilateral purulent otorrhea, weight below the fifth percentile,

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splenomegaly, hepatomegaly, and palpable cervical lymph nodes. His absolute lymphocyte count was 1859 cells/ μ L (normal, 3400-9000 cells/ μ L), and he had no evidence of anemia, thrombocytopenia, or neutropenia. Serum immunoglobulin levels were significantly low (undetectable IgA and IgG), but the serum IgM level was normal (see Table E1 in this article's Online Repository at www.jacionline.org).

Family history revealed no early infant demise, immunodeficiency, cystic fibrosis, or consanguinity. Results of maternal HIV serology were negative during the second trimester but not performed at delivery; the infant had a negative HIV DNA PCR test result. He had 2 full biologic male siblings who enjoyed good health. The infant underwent bronchoscopy and endoscopy, and cytomegalovirus (CMV) was isolated by means of quantitative PCR testing with 500 copies/mL and 8000 copies/mL in the lung and bowel, respectively. His serum CMV concentration was more than 1.4 million copies/mL, but the retinal examination revealed no chorioretinitis. EBV, herpes simplex virus, and CMV serologic

study results were all negative before the onset of immunoglobulin replacement. His lymphocyte phenotyping was remarkable for lymphocytopenia, selective CD4 T-cell lymphocytopenia of 11% (normal range, 31% to 56%), and an absolute count of 212 cells/ μ L (normal range, 1430-4300 cells/ μ L; see Table E1). Complete lymphocyte analysis by means of flow cytometry demonstrated low values of naive CD4 T cells and absent HLA-DR expression on PBMCs. The T-cell receptor excision circle (TREC) analysis of the patient's PBMCs performed on a neonatal dried blood spot in storage and performed at 7 months yielded a normal value of greater than 252 copies/ μ L whole blood. *In vitro* proliferative responses to mitogens were normal, but he had no response to specific antigens (neither *Candida* species nor tetanus), despite previous exposures.

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REVIEW

Technology can be an enormous aid to clinicians in diagnosing serious diseases, as seen in the clinical vignette just presented. However, clinicians must educate themselves in the proper use of technology to take advantage of its power to assist in diagnosis. With the growing awareness of the need to diagnose severe combined immunodeficiency (SCID) in newborn infants, clinicians must become aware of certain disorders that might confound the diagnosis of SCID.^{E1-E4} A very important confounding diagnosis of SCID is vertically acquired HIV infection. These 2 disorders are similar with regard to their early predisposition to serious infection, particularly of fungal origin, such as *Pneumocystis jiroveci* pneumonia and early fatal outcome.^{E5,E6} Their origins are entirely separate, with SCID being the result of monogenic mutations in critical proteins and transcription factors, and HIV infection being the result of the HIV virion attaching to the CD4 receptor and the chemokine receptor CCR5 on monocytes/macrophages or the CXCR4 chemokine receptor on T cells and turning these cells into HIV virion factories. The urgency behind the movement to adopt neonatal screening for SCID with the TREC assay has been beneficial for infants with SCID because of the promise of early corrective hematopoietic stem cell transplantation (HSCT).^{E7-E9} However, only 7 states and 1 US territory have implemented neonatal screening for SCID, and in most cases SCID becomes clinically apparent beyond neonatal age during the first year of life. Thus HIV must remain in the differential diagnosis of SCID, and it is important for clinicians to understand how to test for SCID versus HIV infection.

Diagnosis of HIV infection in children

Because the CD4 T cell is the target of HIV infection, very soon after the birth of a child, there is a massive depletion of CD4 T cells, which is particularly observed in the gastrointestinal lymphoid tissue.^{E10} Cytotoxic T cells specific for HIV antigens develop but soon wane and begin to decrease in number through mechanisms of apoptosis and autophagy.^{E11} With the universal testing of pregnant women for HIV infection in developed countries, infants born to HIV-infected women will have the benefit of suspicion of HIV infection and universal HIV testing. These tests cannot be antibody based because maternal antibodies can persist in the infant born of an HIV-infected mother for up to 18 months. A nucleic acid HIV DNA test is preferred.^{E12} There are pregnant female patients who acquire HIV infection within 3 weeks of their delivery who might have a negative HIV antibody test at the birth of the infant. With mothers with known HIV infection, the chance of such infants acquiring HIV infections is less than 1%, if the mother received appropriate antiretroviral therapy during pregnancy, especially in the last trimester of pregnancy. In underdeveloped countries in which universal anti-HIV prophylactic medications are not given to pregnant women to prevent HIV transmission to their infants, the risk of the infants acquiring HIV infection is at least 10-fold higher (ie, 20% to 30%) and higher still if the infant is breast-fed.^{E13}

Diagnosis of SCID and other T-cell deficiencies in children

Unless there is a previous child in the family with a T-cell immunodeficiency and in the absence of universal screening for SCID with the TREC or another assay, infants might not come to a proper diagnosis of a serious immunodeficiency (primary or secondary) until an opportunistic infection occurs and the infant is

referred to a clinical immunologist. Evaluation of children for primary immunodeficiency is a multistep process, starting with taking a history of the medical problem, performing a physical examination, and ordering a limited number of laboratory screening tests for the structure and function of components of the immune system.^{E14-E16} These tests would include determination of serum IgG, IgA, IgM, and IgE levels; determination of specific antibody responses to recall antigens; complete blood count and differential cell determination; and possibly delayed hypersensitivity skin testing. The next step would include determination of PBMC subsets and lymphocyte proliferation to mitogens and antigens. A third step in performing tests is to perform genetic sequence tests looking for single gene mutations that are associated with specific diagnoses of immunodeficiency. It must be emphasized that because of the fragility of patients with serious forms of immunodeficiency (SCID or other T-cell deficiencies), these evaluations need to be initiated as soon as possible. Table E2 lists the expected results of these evaluations in children with a diagnosis of SCID versus HIV infection. Starting with detection of HIV infection in the infant, the maternal history of HIV infection is critical. In the United States almost all pregnant women are tested for HIV infection either during pregnancy or at the time of delivery by using an antibody test enzyme immunoassay.^{E17} Even if test results are negative, a mother could still be infected if infection occurred within 3 weeks of delivery of her infant because of the time it takes for sensitization of B cells and production of anti-HIV antigen antibodies. HIV infection can be presumptively ruled out by 2 HIV DNA PCR tests performed at greater than 14 days and greater than 4 weeks in a non-breast-fed infant.^{E12-E14} If the infant's mother is HIV infected, these infectivity tests in the infant are extremely important.^{E12} With infants with SCID, both the mother's and infant's HIV test results will prove negative. A chest radiograph might suggest the presence of *Pneumocystis jiroveci* in patients with either SCID or HIV infection because of early immune compromise. A child with HIV infection might have a picture of lymphoid interstitial pneumonia or nodular lymphoid hyperplasia.^{E18} A thymic shadow is usually absent in the SCID condition, whereas usually a thymic shadow can be seen in patients with HIV infection unless HIV disease progression and severe respiratory tract infection have stressed the thymus. The white blood cell count is usually within normal limits for both patients with SCID and those with HIV infection, but the total lymphocyte count is decreased in patients with SCID and mostly normal in patients with HIV infection. Serum immunoglobulin levels are very low in patients with SCID (IgG levels can be normal at birth because of maternal transfer but are reduced by 50% each month because of half-life decay) and very high in patients with HIV infection (IgG, IgA, and IgM), one of the early tests used to differentiate the 2 conditions.^{E19} Polyclonal stimulation by HIV antigens increases the levels of serum immunoglobulins well above the age-matched control values.^{E20} Serum IgE levels are increased in several T-cell deficiencies because of immune dysregulations (eg, hyper-IgE syndrome) and also in patients with HIV infection. Specific antibody function is virtually absent in patients with SCID and greatly reduced in patients with HIV infection, especially in those patients with advanced HIV disease. The CD4 T-cell count being low is a hallmark sign of HIV infection, with an increased number of CD8 T cells per microliter. Infants with SCID typically have both CD4 and CD8 T lymphocytopenias with a compensatory percentage increase in B-cell numbers. HIV-infected infants might also have

a minimal increased percentage of B cells. Lymphocyte proliferation to mitogens and antigens is greatly reduced in patients with SCID and normal to low in patients with HIV disease progression. DNA testing reveals various monogenic mutations in patients with the various types of SCID, but several specific mutations and gene polymorphisms have been detected in patients with HIV infection that modify HIV infection and the speed of HIV disease progression (eg, CCR5Δ32 gene deletion^{E21}). As shown in this clinical vignette, because a low CD4 T-cell count can be seen in either a primary or secondary immunodeficiency, a proper HIV test will rule out the possibility of HIV infection and will allow the clinician to focus exclusively on primary immunodeficiencies.

THE CASE REVISITED

This patient's presenting clinical symptoms of hypogammaglobulinemia, CMV viremia, selective CD4 depletion, poor *in vitro* lymphoproliferation responses to specific antigens, and lack of HLA expression on PBMCs suggested a diagnosis of MHC class II deficiency. The diagnosis was confirmed by more extensive flow cytometry testing for HLA-DQ and HLA-DP expression (none was found). Without extensive flow cytometric analysis of PBMCs, the clinical presentation could have been consistent with a secondary immune-deficient state caused by perinatal HIV infection, especially in light of a normal TREC analysis. This case highlights for the clinical immunologist the importance of how appropriate selection and interpretation of diagnostic assays can distinguish primary and secondary immune deficient states. Flow cytometric analysis beyond simple CD3, CD4, and CD8 cell determinations was essential to assist with this diagnosis. Phenotypic lymphocyte expression of B-cell and natural killer cell populations can assist the clinical immunologist in predicting specific mutations.^{E5,E15} In this case lack of HLA expression on PBMCs suggested thymic epithelial involvement with defective maturation of CD4 cells. Exclusion of secondary immune-deficient states, specifically congenital infections, such as HIV or CMV, require knowledge of the appropriate use of nonserologic testing. In this case with profound hypogammaglobulinemia, all viral serologic test results were negative before passive immunization with IgG supplementation. If only serologic tests had been selected, HIV infection could not be excluded, and active CMV infection would have been missed. Viral DNA testing for HIV and CMV was essential to assist with the diagnosis and development of correct clinical care. The diagnosis of MHC class II deficiency required careful interpretation of available immune technology, including TREC analysis, expanded flow cytometry of PBMCs, and *in vitro* lymphoproliferative responses. A matched related donor HSCT corrected HLA expression on PBMCs, but the CD4 cell count decrease persisted because of a severe thymic defect. HSCT coupled with appropriate antiviral therapy eradicated CMV infection, and the child is doing well at 4 years of age and off all medications (unpublished observation), a finding that stands against the general impression that HSCT is less favorable in patients with MHC class II deficiency.^{E5} The inability of the normal numbers of CD8 T cells to combat serious viral infection in this disorder has not been fully explained.

In summary, this case presentation highlights the need for neonates with suspected SCID or serious T-cell deficiencies to be evaluated for congenital immune system deficiencies but also neonatal HIV infection, which clinically can mimic these disorders. This case demonstrates very clearly that not all T-cell deficiencies can be detected by using the screening TREC assay

and that the full armamentarium of immunologic and genetic testing must be used to arrive at a correct diagnosis. Screening for the SCID disorder with the TREC assay is a remarkable advance, but final diagnosis still involves additional technology.

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REFERENCES

- E1. Buckley RH. The long quest for neonate screening for severe combined immunodeficiency. *J Allergy Clin Immunol* 2012[Epub ahead of print].
- E2. Puck JM. Laboratory technology for detection of SCID neonates: lower T cells vs. genetic lesions. *J Allergy Clin Immunol* 2012[Epub ahead of print].
- E3. Verbsky J, Taker M, Routes J. The Wisconsin experience in newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* 2012 [Epub ahead of print].
- E4. Shearer WT. Screening for severe combined immunodeficiency in newborns. *J Allergy Clin Immunol* 2012[Epub ahead of print].
- E5. Notarangelo LD. Primary immunodeficiencies. *J Allergy Clin Immunol* 2010; 125(suppl):S182-4.
- E6. Chinen J, Shearer WT. Secondary immunodeficiencies, including HIV infection. *J Allergy Clin Immunol* 2010;125(suppl):S195-203.
- E7. Patel NC, Chinen J, Rosenblatt HM, Hanson IC, Krance RA, Paul ME, et al. Outcomes of patients with severe combined immunodeficiency treated with hematopoietic stem cell transplantation with and without preconditioning. *J Allergy Clin Immunol* 2009;124:1062-9, e1-4.
- E8. Railey MD, Lokhnygina Y, Buckley RH. Long-term clinical outcome of patients with severe combined immunodeficiency who received related donor bone marrow transplants without pre-transplant chemotherapy or post-transplant GVHD prophylaxis. *J Pediatr* 2009;155:834-40.
- E9. Gennery AR, Slatter MA, Grandin L, Taupin P, Cant AJ, Veys P, et al, on behalf of members of the Inborn Errors Working Party of the European Group for Blood and Marrow Transplantation and European Society for Immunodeficiency. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? *J Allergy Clin Immunol* 2010;126:602-10.
- E10. Brechley JM, Price DA, Douek DC. HIV disease: fallout from a mucosal catastrophe? *Nat Immunol* 2006;7:235-9.
- E11. Ganesan A, Chattopadhyay P, Brodie T, Qin J, Gu W, Masciola JR, et al. Immunologic and virologic events in early HIV infection predict subsequent rate of progression. *J Infect Dis* 2010;201:272-84.
- E12. Branson BM. The future of HIV testing. *J Acquir Immune Defic Syndr* 2010; 55(suppl):S102-5.
- E13. Shearer WT. Breastfeeding and HIV infection. *Pediatrics* 2008;121:1046-7.
- E14. Chinen J, Paul ME, Shearer WT. Approach to the evaluation of the immunodeficient patient. In: Rich RR, Fleisher TA, Shearer WT, Schroeder H, Kotzin B, editors. *Clinical immunology: principles and practice*. 4th ed. London: Elsevier; 2012. p. 31.
- E15. Oliveira JB, Fleisher TA. Laboratory evaluation of primary immunodeficiencies. *J Allergy Clin Immunol* 2010;125(suppl):S297-305.
- E16. Fried AJ, Bonilla FA. Pathogenesis, diagnosis, and management of primary antibody deficiencies and infections. *Clin Microbiol Rev* 2009;22:396-414.
- E17. Panel on Antiretroviral Therapy and Medical Management of HIV-infected Children. Guidelines for the use of antiretroviral agents in pediatric HIV infection. August 11, 2011; p 12. Available at: <http://aidsinfo.nih.gov/ContentFiles/PediatricGuidelines.pdf>. Accessed February 2, 2012.
- E18. Zar HJ. Chronic lung disease in human immunodeficiency virus (HIV) infected children. *Pediatr Pulmonol* 2008;43:1-10.
- E19. Starr SE. Immunopathogenesis of HIV infection. In: Shearer WT, Hanson IC, editors. *Medical management of AIDS in children*. Philadelphia: Sanders; 2005. p. 53-68.
- E20. Moir S, Fauci AS. Pathogenic mechanisms of B-lymphocyte dysfunction in HIV disease. *J Allergy Clin Immunol* 2008;122:12-9.
- E21. Hutter G, Nowak D, Mossner M, Ganepola S, Musig A, Allers K, et al. Long-term control by CCR5 Delta 32/Delta 32 stem-cell transplantation. *N Engl J Med* 2009;360:692-8.
- E22. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatrics AIDS Clinical Trials Group P1009 Study. *J Allergy Clin Immunol* 2003;112:973-80.

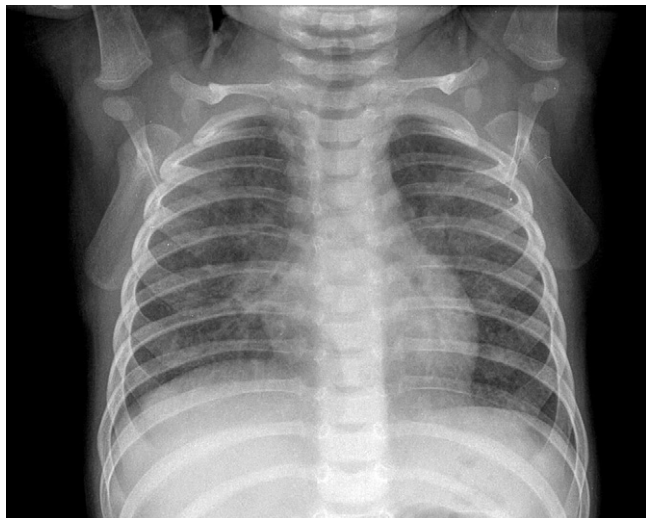


FIG E1. Chest radiograph of a 7-month-old boy with cough, fever, and failure to thrive.

TABLE E1. Summary of clinical studies performed before IgG supplementation or HSCT

Test	Case	Age-matched healthy control subjects*
Absolute lymphocyte count	1,859 cells/ μ L	3,400-9,000 cells/ μ L
Serum IgG	<7 mg/dL	218-907 mg/dL
Serum IgA	<7 mg/dL	10-85 mg/dL
Serum IgM	78 mg/dL	31-116 mg/dL
CMV IgG	Negative	Negative
CMV PCR	>1.4 $\times$ 10 ⁶ copies/mL	Not detected
HIV enzyme immunoassay	Negative	Negative
HIV DNA PCR	Undetectable	Not detected
CD3 T-cell percentage; count	46%; 815 cells/ μ L	49% to 76%; 1,900-5,900 cells/ μ L
CD4 T-cell percentage; count	11%; 212 cells/ μ L	31% to 56%; 1,400-4,300 cells/ μ L
CD4 ⁺ CD45RA ⁺ (naive) T-cell percentage; count	2.5%; 45 cells/ μ L	64% to 93%; 1,100-3,600 cells/ μ L
CD8 T-cell percentage; count	34%; 635 cells/ μ L	12% to 24%; 500-1,700 cells/ μ L
CD19 B-cell percentage; count	51%; 947 cells/ μ L	14% to 37%; 610-2,600 cells/ μ L
CD3 ⁺ DR ⁺ (activated) T-cell percentage; count	<0.2%; 3 cells/ μ L	6% to 38%; 90-550 cells/ μ L
Lymphocyte proliferations		
Mitogens	100% of normal control values for PHA, ConA, and PWM	PHA: 165,549-307,650 cpm ConA: 93,548-245,877 cpm PWM: 42,905-138,816 cpm
Antigens	<i>Candida</i> species: <2,000 cpm Tetanus: <2,000 cpm	>2,000 cpm >2,000 cpm
TREC [†]	>252 Copies of TREC/ μ L whole blood	$\geq$ 252 Copies of TREC/ μ L whole blood

ConA, Concanavalin A; PWM, pokeweed mitogen.

*Flow cytometric control data (10th-90th percentiles) were taken from Shearer et al.^{E22} The lymphocyte proliferation control data were taken from 20 adult laboratory volunteers, Texas Children's Hospital, Houston, Tex.

[†]Performed on neonatal dried blood spots with parental consent as part of a US Centers for Disease Control-funded pilot study of SCID newborn screening by the Texas Department of State Health Services.

TABLE E2. Evaluation of SCID and other T-cell deficiencies versus HIV infection in infants

Test or procedure	SCID/other T-cell deficiencies	HIV
Mother's HIV test (if known)	Negative/negative	Positive unless mother infected within 3 wk of birth of infant
Infant's HIV test	Negative/negative	Positive by HIV DNA test
Chest radiograph	PJP/PJP	PJP; LIP
Thymic shadow	Absent/absent	Present unless rapid HIV progression
White blood cell count	Normal/normal	Normal to increased
Lymphocyte count	Low/low	Normal
TREC number	Low/variable	Undetermined, anecdotal evidence indicates normal
Serum immunoglobulins	Low except for maternal IgG and IgE (some cases)/variable	High 2× to 3× normal
Specific antibodies	Low/low	Low with HIV disease progression
CD4 T-cell count	Low/variable	Low
CD8 T-cell count	Low/variable	High
Lymphocyte proliferations		
Mitogens	<10% of normal control values/variable	Normal to low depending on disease progression
Antigen	<10% of normal control values/variable	Normal to low
DNA testing	Specific mutations that cause SCID/same	Mutations and polymorphisms associated with HIV disease progression

LIP, Lymphoid interstitial pneumonitis; PJP, *Pneumocystis jiroveci* pneumonitis.