

The ‘Five Fingers’ of the Diagnostic Evaluation for Suspected Immunodeficiency

Ashvini Varadhi, MD; Joseph R. Hageman, MD; and Karl O. A. Yu, MD, PhD

The human immune system can be divided into two main categories: innate and adaptive. Innate immunity is a primitive system that does not require prior exposure and is activated by microbial products, such as lipopolysaccharide, cell wall components, and certain nucleotides. Natural barriers of the body such as the skin, respiratory tract, and gastrointestinal mucosa, as well as the reticuloendothelial function of the liver and

Ashvini Varadhi, MD, is a Resident, Department of Pediatrics, Comer Children's Hospital, Pritzker School of Medicine, University of Chicago. Joseph R. Hageman, MD, is Senior Clinician Educator, Comer Children's Hospital, Pritzker School of Medicine, University of Chicago; and Emeritus Attending Physician, NorthShore University HealthSystem. Karl O. A. Yu, MD, PhD, is a Fellow, Section of Infectious Diseases, Department of Pediatrics, Comer Children's Hospital, Pritzker School of Medicine, University of Chicago.

Address correspondence to: Ashvini Varadhi, MD, Comer Children's Hospital, 5721 S. Maryland Avenue, Chicago, IL 60637; email: ashvini.varadhi@uchospitals.edu.

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spleen, are where innate immunity are most relevant. Phagocytic cells such as granulocytes, monocytes, macrophage, and natural killer (NK) cells are activated and use the secretion of cytokines, chemokines, and other soluble mediators as the main functional components of innate immunity.

Complement is also activated by microbial products and works in conjunction with the phagocytic cells to clear pathogens. Phagocytic defects present with recurrent bacterial and fungal infections involving skin or soft tissue and other organs. These are principally caused by staphylococci, certain gram-negative bacteria (eg, *Salmonella*, *Serratia*, *Klebsiella*, *Burkholderia*), fungi, and mycobacteria. Complement deficiencies may present with recurrent or serious infections — particularly sepsis or meningitis — caused by encapsulated bacteria like *Streptococcus pneumoniae* and *Neisseria* species, as well as with autoimmune diseases such as systemic lupus erythematosus or glomerulonephritis.^{1,2}

Adaptive immunity, in contrast, requires an inducing antigen that directs a specific immune pathway response and preserves immunologic memory. Conventional T lymphocytes providing cellular immunity are activated after exposure to peptide fragments of antigen that are bound to cell surface human leukocyte antigen molecules. Deficiencies in cell-mediated immunity may present with failure to thrive or opportunistic infections. These would include pneumonia caused by pyogenic bacteria, viruses, and *Pneumocystis jirovecii*, gas-

trointestinal illnesses caused by viruses and protozoans, and skin or mucous membrane fungal infections.

B cells provide humoral immunity through activation and release of specific immunoglobulin against pathogens. Deficiencies in humoral immunity present as recurrent sinopulmonary infections with encapsulated bacteria and viruses, repeated enteric illnesses caused by enteroviruses or *Giardia*, or a failure to respond against infection with proper use of antibiotics. Autoantibody-mediated autoimmune disease and inflammatory bowel disease may also be seen.^{1,2}

A number of reviews and updates of primary immunodeficiencies and the infections with which these may present are available. In the past decade, there has been an increase in the number of primary immunodeficiencies for which genetic causes have been defined.¹⁻⁵

THE “FIVE FINGERS” OF THE IMMUNE EVALUATION Cell Number

Pediatricians should be able to identify immunodeficiency warning signs. One helpful guide is the “10 Warning Signs for Primary Immunodeficiency” published by the Jeffrey Modell Foundation⁶ (see Sidebar, page 211). A cost-effective and informative first step is the complete blood count (CBC) with differential and platelet count. One must review the absolute neutrophil count (total white blood cell count x percentage of segmented + band and other forms) and absolute lym-

phocyte count and compare these with age-specific limits in standard references to screen for neutropenia and lymphopenia, respectively.^{7,8} Repeated CBCs may be needed to help distinguish bona fide abnormalities from a “blip.” As an example, both severe congenital neutropenia and cyclic neutropenia have patterns that must be confirmed with several CBCs in a row.

This stands in contrast to immunocompetent children, who may be neutropenic or lymphopenic when they are dealing with a viral infection, but would have normal blood counts when they are at baseline. Review of the blood smear for cell morphology may lend clues to a diagnosis as well. For instance, Wiskott-Aldrich syndrome will present with thrombocytopenia with small platelets. Howell-Jolly bodies are seen in anatomic or functional asplenia.²

Flow cytometry for lymphocyte subset analysis (commonly ordered as “T/B cell counts” or “lymphocyte subsets” in laboratories) provide information regarding the adaptive immunity of the patient. Flow cytometry should be considered a reflexive test if there is demonstrated, unexplained lymphopenia, as this is a straightforward screen to determine deficiency in the number of CD4 T cells (helper T cells), CD8 T cells (cytotoxic or killer T cells), B lymphocytes, and NK cells.

B cells are identified by expression of CD19 or CD20. NK cells are defined by the expression of CD16 and/or CD56, with the absence of the T cell co-receptor CD3. The absolute number of each lymphocyte subset is the more important measure and should be compared to age-specific norms. However, in the setting of transient lymphopenia due to intercurrent illness, confirmation of a normal percentage of each cell subset is reassuring.

In addition to an initiated evaluation by the pediatrician, a screen for lymphopenia may be included in many state newborn screens (Guthrie or PKU cards). Since 2008, Wisconsin has been screening new-

born blood for levels of T cell receptor excision circles (TRECs). TRECs are the by-products of genomic DNA recombination in recently developed T cells and thus act as a screen for severe T lymphopenia, such as in severe combined immunodeficiency and 22q11.2 deletion syndrome.

Kappa-deleting recombination excision circles (KRECs) are the analogous products formed by B cells during development and may serve as a newborn screen for diseases causing profound B lymphopenia (eg, X-linked agammaglobulinemia). TREC screening is now available in an increasing number of states, whereas KREC screening is in pilot programs. For the primary care provider confronted with an abnormal TREC or KREC screen, prompt discussion with an immunologist is needed.⁹

Most patients seen in the primary care setting will have a minimal history of infections, appropriate responses to anti-infective therapy, no history of failure to thrive, and no family history of immunodeficiency. In these cases, a normal CBC with differential, with or without a lymphocyte subset study, may be sufficient laboratory evaluation. A broader evaluation may be pursued if and when the patient presents with a more impressive clinical picture.

Cell Function

Sometimes, despite normal cell numbers, functionality of cells may be impaired in a patient with immunodeficiency. Evaluating lymphocyte and neutrophil function may provide further information. T-cell function can be tested both in vivo and in vitro. In vivo testing involves demonstrating delayed-type hypersensitivity to select antigens. The most common test involves the intradermal injection of *Candida* antigen (0.1 mL of standardized solution), which should elicit an area of induration in most patients in 48 to 72 hours. This represents the hypersensitivity reaction instituted by CD4 T cells in response to antigen processing and presentation.

In vitro cell function evaluation consists of several other tests. Testing for cel-

SIDEBAR

The 10 Warning Signs of Primary Immunodeficiency

1. Four or more new ear infections within 1 year.
2. Two or more serious sinus infections within 1 year.
3. Two or more months on antibiotics with little effect.
4. Two or more pneumonias within 1 year.
5. Failure of an infant to gain weight or grow normally.
6. Recurrent, deep skin or organ abscesses.
7. Persistent thrush in mouth or fungal infection on skin.
8. Need for intravenous antibiotics to clear infections.
9. Two or more deep-seated infections including septicemia.
10. A family history of primary immunodeficiency.

From the Jeffrey Modell Foundation.⁶

lular proliferation against different mitogens and antigens is a good general screen. This used to be done by measuring [³H] thymidine incorporation by proliferating leukocytes in response to various stimuli. Many laboratories have now switched to using flow cytometric methods to detect incorporation of nucleotide probes, such as 5-ethynyl-2'-deoxyuridine. Pokeweed mitogen, a plant lectin, stimulates both B and T cells. Phytohemagglutinin and concanavalin A, on the other hand, stimulate T cells.

Cellular proliferation against specific antigens, such as *Candida*, tetanus, or cytomegalovirus, may also be done using the same methods. These should be done only in a previously immunized or infected patient. In many laboratories, these tests are ordered as “lymphocyte proliferation” or “mitogen/antigen proliferation” tests.

Lastly, oxidation of the indicator molecule dihydrorhodamine-123 by in vitro-activated neutrophils is the test-of-choice for neutrophil function (ie, the neutrophil

oxidative burst) and is the screen for chronic granulomatous disease. Because of its higher sensitivity and better reproducibility, this test has replaced the previously used nitroblue tetrazolium assay.

Attention should be paid in using in vitro tests of cellular function, since results are dependent on the viability of blood specimens. For instance, dihydrorhodamine-123 oxidation testing necessitates receipt of the specimen by the laboratory within 48 hours, shipment at room temperature, and requires co-shipment of a normal control. We advise checking with one's local laboratory for specimen handling and shipment requirements.

Immunoglobulin Levels (“Number”)

Most humoral immunodeficiencies present with normal B-cell numbers, so measurement of the immunoglobulin levels is needed in the diagnosis of B-cell defects. Immunoglobulin may first be roughly quantified by looking at the protein-albumin gap. A narrow total protein-albumin gap (hence, a low globulin level) is an immediate “flag” for possible hypogammaglobulinemia (IgG < two standard deviations of the mean, adjusted for age), or even agammaglobulinemia (IgG < 100 mg/dL).

With a higher suspicion for immunodeficiency, the antibody should be quantified within the various isotypes. Low levels of IgG, IgA, or IgM may be suggestive of a number of humoral immunodeficiencies. Measurement of subclasses of IgG may reveal isolated IgG subtype deficiencies. As with cell number, these should be interpreted with the appropriate age-related normal intervals.^{10,11} Elevated levels of IgM may suggest hyperimmunoglobulin M syndrome, a primary immunodeficiency involving T-helper type 2 cells and B-cell differentiation. Elevated IgE levels may be due to atopy and allergic disease, or may be a sign of Job's syndrome, given the appropriate findings and history. Elevated IgG levels are seen in some autoimmune diseases and in mycobacterial infection.

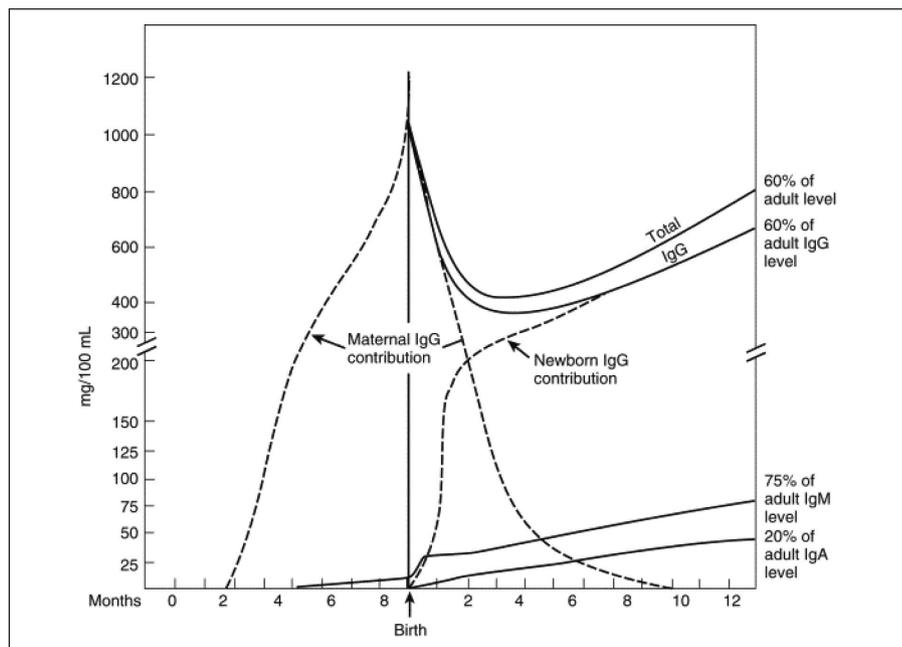


Figure 1. Antibody levels in the fetus and in infancy. Immunoglobulin (Ig) G in the fetus and newborn is normally of maternal origin, which is then slowly replaced by the child's endogenous IgG with time. IgM and IgA, on the other hand, are produced by the child. (Reproduced from Lewis DB, Tu W;¹⁴ with permission.)

Antibody Function

Antibody function is evaluated through the measurement of antibody titers against specific vaccines or infection. Antibodies against diphtheria and tetanus toxins evaluate responses against vaccination with conventional T-dependent protein antigens. Titers against measles, mumps, rubella, or varicella measure the response against virus vaccination. Antibody tests against *Haemophilus* and pneumococcal polysaccharides traditionally test the response against T-independent type 2 antigens — although this is now somewhat clouded due to increasing use of conjugate vaccines. Conventionally, these tests are done at least 4 weeks post-vaccination.

Serology and other tests against certain pathogens may also be included in the immunodeficiency workup. One should have a low threshold for HIV testing, especially in the setting of opportunistic infections or failure to thrive. HIV antibody testing by enzyme-linked immunosorbent assay (ELISA) is the screen of choice for patients who have normal antibody levels, or for neonates and young infants (as this

would test the mother's HIV status and the child's risk for HIV vertical acquisition). Direct detection of HIV (either by viral load or by proviral DNA polymerase chain reaction [PCR]) should be done if suspicion is high, or if acute antiretroviral syndrome is suspected. Testing for antibody responses to other common viral infections, like Epstein-Barr virus, cytomegalovirus, and parvovirus, may be called for in the appropriate setting.

One needs caution in interpreting tests for antibody function in infants and neonates who are born at term, as trans-placentally acquired maternal IgG comprises the bulk of the child's IgG until at least 4 months of age, and these slowly decrease with time. Similarly, premature infants will have lower levels of IgG, as most maternal IgG crosses the placenta in the third trimester (see Figure 1). As the antibody response to T-independent type 2 antigens (eg, pneumococcal polysaccharides) is immature in infants due to a relative deficit of splenic marginal zone B cells, measurement of antipneumococcal antibody is less likely to be useful and should be interpreted with caution for patients younger than 18 to 24

months.^{2,12} Lastly, antibody function and IgG levels should be carefully interpreted in patients who have recently received exogenous immunoglobulin — either as pre- or post-exposure prophylaxis, or for therapy, as in Kawasaki disease.

Complement System Studies

After evaluation of the first four fingers, complement studies may help with further investigation of patients with recurrent infections with normal levels and function of leukocytes, lymphocyte subsets, and antibody. Although primary complement deficiencies are rare, evaluation for these is warranted for patients with recurrent or serious infection with certain encapsulated organisms, including *S. pneumoniae*, *S. pyogenes* and *Neisseria* species.

The total hemolytic complement assay (CH₅₀) is the initial screening test for complement deficiency or defects. A CH₅₀ level that is reduced or zero warrants further evaluation with measurement of individual complement levels. The alternate pathway (AH₅₀) screening test can be used when the CH₅₀ assay is normal but complement deficiency is still suspected.

Complement deficiency may either be primary or acquired. In the setting of infection, especially post-streptococcal or viral-associated glomerulonephritis, complement levels may be decreased. An abnormal CH₅₀ or AH₅₀ result when the patient is sick or admitted in the hospital merits a repeat test when the patient has recovered. Hypocomplementemia due to consumption is also seen in autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis.¹³

CASES UTILIZING THE FIVE FINGERS OF EVALUATION

Case 1

History

A 3-week-old, full-term male infant presented to his primary pediatrician with a 2-day history of a perianal abscess. He was afebrile and his review of systems was otherwise negative. There is no family his-

tory of immunodeficiency or recurrent infections.

Admission Labs/Procedure

White blood cell count was 13,600 cells/mm³. Differential: segmented neutrophils 37%, lymphocytes 36%, monocytes 19%, and eosinophils 7%. Absolute neutrophil count was 5,032/mm³, absolute lymphocyte count was 4,896/mm³. C-reactive protein was < 3 mg/L.

An incision and drainage was performed with wound cultures returning positive for an extended-spectrum beta-lactamase-producing *E. coli*. The organism was sensitive to amikacin, gentamicin, ertrapenem, imipenem, and tigecycline, and resistant to ampicillin/sulbactam, cefepime, ceftriaxone, ciprofloxacin, moxifloxacin, and trimethoprim/sulfamethoxazole.

The infection improved after the incision and drainage, and the patient received 10 days of intravenous meropenem and gentamicin.

Evaluation Review

Although a very resistant organism caused the infection, this appeared to be a soft tissue infection that responded to the appropriate surgical and anti-infective intervention. No further workup was done beyond the CBC with the differential, and confirmation that the child had a negative HIV antibody screen. Measuring other immunoglobulins is of little utility in this age, since most of the baby's antibody is transplacentally acquired maternal IgG.

Case 2

History

A 3-year-old boy with no significant medical history presented with a 2-day history of fever and right shoulder pain. His exam was notable for pain and swelling over his right shoulder and decreased range of motion. An MRI showed a fluid collection anterior to and posterior to his scapula, and soft tissue edema in his biceps, triceps, and his rotator cuff.

Admission Labs/Management

White blood cell count was 20,100/mm³. Differential: segmented neutrophils 79%, bands 3%, lymphocytes 13%, monocytes 5%. Absolute neutrophil count was elevated at 16,482/mm³; the absolute lymphocyte count was 2,613/mm³. C-reactive protein was 134 mg/L. ESR was 60 mm/h.

The boy was found to have bacteremia, pyomyositis and scapular osteomyelitis due to methicillin-sensitive *S. aureus*. His repeat blood culture turned negative and his fevers stopped by day 3 of intravenous oxacillin and gentamicin. His shoulder pain improved with analgesics and surgical debridement of the area.

Labs on Hospital Day 4

White blood cell count was 11,700/mm³. Differential: segmented neutrophils 80%, bands 2%, lymphocytes 10%, monocytes 8%. Absolute neutrophil count was 9,594/mm³, absolute lymphocyte count was 1,170/mm³. C-reactive protein was 29 mg/L. Protein/albumin gap was 4.1 g/dL.

The patient completed 4 weeks of intravenous antibiotics (oxacillin with gentamicin, then cefazolin on discharge), and continued with 2 more weeks of oral cephalexin. His C-reactive protein normalized on day 21 of therapy.

Evaluation Review

This clinical presentation is very impressive, but the level of infective involvement is not beyond the realm that is expected for epidemic *S. aureus*. An isolated, one-time history of osteomyelitis or septic arthritis is commonly seen in the immunocompetent pediatric population and therefore is not necessarily a trigger for a broad immunodeficiency evaluation. A normal CBC with differential and protein/albumin gap is sufficient in the absence of additional history of serious or recurrent infections.

Case 3

History

An otherwise healthy 7-year-old boy has a history of recurrent otitis media

since age 1 year. He usually presented with otalgia, sometimes with low-grade fever, and would improve with antibiotics. His ear infections decreased in frequency when tympanostomy tubes were placed and recurred when his ear tubes fell out. Recently, he presented with purulent ear drainage that grew *Aspergillus fumigatus* in culture. His symptoms improved, and a repeat culture of ear fluid was negative after a course of otic amphotericin B.

Outpatient Laboratory Studies

Cell counts: The CBC showed a white blood cell count at 7,000/mm³, with a differential of granulocytes 33%, lymphocytes 53%, monocytes 8%, eosinophils 5%. Absolute neutrophil count was 2,310/mm³, absolute lymphocyte count was 3,710/mm³.

Flow cytometry: CD4 T cells 1,006/mm³, CD8 T cells 811/mm³, B cells 744/mm³, NK cells 150/mm³. Absolute lymphocyte count by flow cytometry was 3,438/mm³.

Immunoglobulin levels: IgG 551 mg/dL, IgA 35 mg/dL, IgM 37 mg/dL (all normal for age). IgE 302 IU/mL (high). IgG subclasses were normal.

Antibody function: *Haemophilus influenzae* IgG 2.4 mg/L (immune). Tetanus toxoid IgG 2.4 IU/mL (immune). Pneumococcal polysaccharides antibody test was deferred.

Evaluation Review

This child’s history is commonly encountered by otolaryngologists. Such patients may warrant a more extensive immunodeficiency evaluation. The absence of a history of other infections beyond the ear suggests that the diagnosis of a primary immunodeficiency may be less likely. This patient’s evaluation is normal, except for the suggestion of an atopic predisposition. The patient’s elevated IgE level, in the absence of more history, is insufficient to warrant genetic testing for Job’s syndrome.

TABLE.

Potential Laboratory Tests for Immunological Evaluation

Fingers of Evaluation	Laboratory Tests
Cell number	CBC with differential Flow cytometry (lymphocyte subsets)
Cell function	In vivo: delayed-type hypersensitivity In vitro: antigen and/or mitogen stimulation, neutrophil oxidative burst
Immunoglobulin levels (“number”)	Protein-albumin gap IgG, IgA, IgM, IgE IgG subtypes
Antibody function	Antibody titers for specific antigens
Complement system studies	CH ₅₀ , AH ₅₀

AH₅₀ = complement activity, alternative pathway; CBC = complete blood count; CH₅₀ = total complement activity; Ig = immunoglobulin.

Case 4

History

A 14-year-old boy with a history of environmental allergies and moderate persistent asthma in early childhood (including 3 hospitalizations and an average of one course of steroids yearly) presented an acute history of bilateral proptosis and green nasal discharge. He was found to have chronic allergic sinusitis due to *Aspergillus flavus*, with erosion through the sinuses, causing mass effect on the brain. He responded with intravenous steroids and surgical debulking. The fungal infection was treated with liposomal amphotericin B and voriconazole. Amphotericin B was discontinued once the patient had therapeutic levels of voriconazole while on oral therapy.

Labs on Admission

Cell count: White blood cell count was 4,200/mm³. Differential: granulocytes 27%, lymphocytes 36%, monocytes 12%, eosinophils 24%.

Absolute neutrophil count was 1,134/mm³, absolute lymphocyte count was 1,512/mm³.

Flow cytometry: CD4 T cells 164/mm³ (low), CD8 T cells 133/mm³, B cells 195/mm³, NK cells 135/mm³. CD4:CD8 ratio 1.2 (low normal). Absolute lymphocyte count by flow cytometry 734/mm³.

The screen was expanded after his initial laboratory studies demonstrated CD4 lymphopenia. A discordance between the lymphocyte count in the CBC and in flow cytometry was noted.

Further Labs

Cell count: White blood cells 12,800/mm³. Differential: granulocytes 79%, lymphocytes 12%, monocytes 9%. Absolute neutrophil count 10,112/mm³, absolute lymphocyte count 1,536/mm³.

Flow cytometry: CD4 T cells 336/mm³ (low), CD8 T cells 310/mm³, B cells 117/mm³, NK cells 63/mm³. CD4:CD8 ratio 1.1 (low normal). Absolute lymphocyte count 914/mm³.

Cell function: Neutrophil oxidative burst (dihydrorhodamine-123 oxidation) was normal. Maximal proliferative response to pokeweed mitogen: 7% of CD45⁺ cells, 15% of CD3⁺ cells (both normal). Maximal proliferative response to phytohemagglutinin: 13% of CD45⁺ cells (low), 30% of CD3⁺ cells (low). Noted increase in spontaneous cell death by day 4 of culture. QuantiFERON-Gold negative, with mitogen control at 5.2 IU IFN-gamma/mL (mid-range).

Immunoglobulin levels: IgG 1,403 mg/dL, IgA 159 mg/dL, IgM 59 mg/dL (normal). IgE 4,060 IU/mL (very high).

Antibody function: HIV antibody

screen negative; *Aspergillus fumigatus* IgE 17 kU/L (class III), IgG > 200 mg/L (high); *H. influenzae* IgG 1.5 mg/L (immune); Tetanus toxoid IgG 0.2 IU/mL (immune); 23-valent pneumococcal polysaccharide IgG: serotype 19 at 2.6 mg/L (low normal), with the 22 other serotypes at < 1.3 mg/L (absent).

Complement: CH₅₀ level at 168 U/mL (normal).

Evaluation Review

With the extent of disease seen, there was a concern for immunodeficiency. The patient’s laboratory results were suggestive of a CD4 T cell-specific defect in number and function, possibly due to an apoptotic pathway problem. The near-absence of polysaccharide-specific antibody also suggests a defect isolated on either B-1 or marginal zone B-cell subpopulations. The differential diagnosis included idiopathic CD4 T lymphopenia, common variable immunodeficiency, selective anti-polysaccharide antibody deficiency, and Job’s syndrome. The patient was referred to Immunology for further evaluation. His relative CD4 T lymphopenia remained persistent, and he remained without an anti-pneumococcal polysaccharide response after immunization. He has recovered from his fungal sinusitis and is now on intravenous IgG therapy as immune replacement, with common variable immunodeficiency as a working diagnosis.

DISCUSSION

Guides for when and how to utilize laboratory testing for suspected immunodeficiency are necessary for general pediatricians. These clinicians are commonly the first to initiate a diagnostic evaluation of a patient. As presented in the cases above, not every patient requires an expensive and complex evaluation. Utilization of the “five fingers” of the immunological evaluation (see Table, page 214) can provide primary care pediatricians with a reference for approaching suspected immunodeficiency and an aid in properly

identifying which patients require further subspecialty follow-up. ■

REFERENCES

1. Fleisher TA. Back to Basics: Primary immune deficiencies: windows into the immune system. *Pediatr Rev.* 2006;27(10):363-371.
2. Lederman HM. Clinical presentation of primary immunodeficiency diseases. In: McMillan JA, Feigin RD, DeAngelis CD, Jones MD, eds. *Oski’s Pediatrics: Principles & Practice.* 4th ed. Philadelphia, PA: Lippincott, Williams & Wilkins; 2006:2441-2444.
3. Carneiro-Sampaio M, Coutinho A. Immunity to microbes: lessons from primary immunodeficiencies. *Infect Immun.* 2007;75(4):1545-1555.
4. Oliviera JB, Fleisher TA. Laboratory evaluation of primary immunodeficiencies. *J Allergy Clin Immunol.* 2010;125(2 Suppl 2):S297-S305.
5. International Union of Immunological Societies Expert Committee on Primary Immunodeficiencies, Notarangelo LD, Fischer A, et al. Primary immunodeficiencies: 2009 update. *J Allergy Clin Immunol.* 2009;124(6):1161-1178.
6. Jeffrey Modell Foundation. Primary Immunodeficiency Resource Center. Available at: <http://www.info4pi.org/jmf/>. Accessed April 18, 2013.
7. Walkovich K, Boxer LA. How to approach neutropenia in childhood. *Pediatr Rev.* 2013;34(4):173-183.
8. Shearer WT, Rosenblatt HM, Gelman RS, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. *J Allergy Clin Immunol.* 2003;112(5):973-980.
9. Verbsky J, Thakar M, Routes J. The Wisconsin approach to newborn screening for severe com-

10. Jolliff CR, Cost KM, Stivirins PC, et al. Reference intervals for serum IgG, IgA, IgM, C3, and C4 as determined by rate nephelometry. *Clin Chem.* 1982;28(1):126-128.
11. Kjellman NM, Johansson SG, Roth A. Serum IgE levels in healthy children quantified by a sandwich technique (PRIST). *Clin Allergy.* 1976; 6(1):51-59.
12. Lewis DB, Wilson CB. Developmental immunology and role of host defenses in fetal and neonatal susceptibility to infection. In: Remington JS, Klein JO, Wilson CB, Nizet V, Maldonado YA, eds. *Infectious Diseases of the Fetus and Newborn Infant.* 7th ed. Philadelphia, PA: Elsevier/Saunders; 2011:83-169.
13. Bonilla FA, Bernstein IL, Khan DA, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *Ann Allergy Asthma Immunol.* 2005;94(5 Suppl 1):S1-S63.
14. Lewis DB, Tu W. The physiologic immunodeficiency of immaturity. In: Stiehm ER, Ochs HD, Winkelstein JA, eds. *Immunologic Disorders in Infants and Children.* 5th ed. Philadelphia, PA: Elsevier/Saunders; 2004:687-760.

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