

Relevance of antibody testing in patients with recurrent infections

Roshini S. Abraham, PhD *Rochester, Minn*

INSTRUCTIONS

Credit can now be obtained, free for a limited time, by reading the review articles in this issue. Please note the instructions listed below:

1. Review the target audience, learning objectives and author disclosures.
2. Complete the pre-test online at www.jacionline.org (click on the On-line CME heading).
3. Follow the online instructions to read the full version of the article, including the clinical vignette and review components.
4. Complete the post-test. At this time, you will have earned 1.00 *AMA PRA Category 1 CME Credit*TM.
5. Approximately 4 weeks later you will receive an online assessment regarding your application of this article to your practice. Once you have completed this assessment, you will be eligible to receive 2 MOC Part II Self-Assessment credits from the American Board of Allergy and Immunology.

Date of Original Release: August 2012. Credit may be obtained for these courses until July 31, 2014.

Copyright Statement: Copyright © 2012-2014. All rights reserved.

Target Audience: Physicians and researchers within the field of allergic disease.

Accreditation/Provider Statements and Credit Designation: The American Academy of Allergy, Asthma & Immunology (AAAAI) is accredited by the Accreditation Council for Continuing Medical Education

(ACCME) to provide continuing medical education for physicians. The AAAAI designates these educational activities for a maximum of 1 *AMA PRA Category 1 Credit*TM. Physicians should only claim credit commensurate with the extent of their participation in the activity.

List of Design Committee Members: Roshini S. Abraham, PhD (author), and James T. Li, MD, PhD (series editor)

Activity Objectives

1. To describe the most commonly used diagnostic laboratory tests for evaluation of humoral immunity (specifically antibody responses).
2. To describe the caveats in interpretation of antibody data and inter-laboratory comparison of test results.
3. To list primary and secondary causes of defective antibody responses.
4. To describe alternate approaches to assessment of antibody function in patients receiving replacement immunoglobulin therapy.

Recognition of Commercial Support: This CME activity has not received external commercial support.

Disclosure of Significant Relationships with Relevant Commercial Companies/Organizations: R. S. Abraham declares that she has no relevant conflicts of interest. J. T. Li has consulted for Abbott.

CLINICAL VIGNETTE

A 39-year-old woman was found to have agammaglobulinemia when being evaluated for recurrent infections by an infectious disease specialist. There was a prior history of chronic sinusitis for at least 5 years and radiographic evidence of right lower lobe pneumonia at the present visit. The patient had an IgG level of 29 mg/dL, an IgA level of less than 1 mg/dL, and an IgM level of 8 mg/dL. In addition to low IgG₁ and IgG₃ subclass levels, IgG₂ and IgG₄ were absent. Additional infectious history included 1 episode of facial shingles, recurrent conjunctivitis and otitis media, cellulitis of the right thigh, and plantar warts on both feet.

On eliciting early history, it became apparent that there was a history of infections in childhood and the teenage years, which became more frequent in the third decade of life. Family history

was unremarkable other than 1 male sibling with recurrent infections.

Radiologic examination of the chest (contrast-enhanced computed tomography) revealed scattered pulmonary nodules and bilateral hilar and mediastinal adenopathy (Fig E1). The nodular infiltrates were progressive over time; however, there were no respiratory symptoms, and thus the nodules were considered as possible sarcoidosis. Results of fungal serology were negative. A right lower lobe lung biopsy revealed acute and chronic inflammation with increased eosinophils and focal pneumonia. There was no evidence of malignancy or granuloma, and the bronchoalveolar lavage fluid was negative for infection by smear and culture but also showed increased eosinophil numbers. A retroperitoneal mass was noted on the left side and was considered a possible lymphadenopathy. Additional work-up did not indicate a lymphoma or pancreatic malignancy. No splenomegaly was observed. Bone marrow analysis revealed polyclonal lymphoid hyperplasia with nodular lesions of benign lymphoid aggregates. A thyroid follicular adenoma was detected and removed surgically. There was no evidence of protein-losing enteropathy or urinary protein loss to account for the hypogammaglobulinemia, and the patient had not been taking steroids, immunosuppressive medications, or other drugs that could potentially cause a secondary hypogammaglobulinemia.

From the Cellular and Molecular Immunology Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic.

Received for publication March 30, 2012; revised June 6, 2012; accepted for publication June 13, 2012.

Corresponding author: Roshini S. Abraham, PhD, Hilton 210e, Mayo Clinic, 200 1st St SW, Rochester, MN 55905. E-mail: abraham.roshini@mayo.edu.
0091-6749/\$36.00

© 2012 American Academy of Allergy, Asthma & Immunology
<http://dx.doi.org/10.1016/j.jaci.2012.06.013>

Additional laboratory analysis included HIV testing, results of which were negative; lymphocyte subset quantitation (T, B, and natural killer cells), results of which were all within normal limits; and isohemagglutinin measurements, results of which were negative. Antibody responses to vaccination was assessed for diphtheria toxoid (DT), tetanus toxoid (TT), *Haemophilus influenzae B*, Hepatitis A and B, cytomegalovirus, EBV, respiratory syncytial virus, rubeola, varicella zoster, and *Streptococcus pneumoniae* polysaccharides. There were low or undetectable responses to all of these antigens, both protein and polysaccharide. Other immunologic analyses included quantitative B-cell subset immunophenotyping, which was

notable for significantly decreased class-switched memory B-cell numbers, as well as decreased plasmablast and increased transitional B-cell numbers. Lymphocyte proliferation to mitogens was modestly decreased, whereas proliferations to antigens (*Candida* species and tetanus toxoid) were normal. Natural killer cell cytotoxic function was also normal. The patient was initiated on immunoglobulin replacement for treatment of the agammaglobulinemia.

The full version of this article, including a review of relevant issues to be considered, can be found online at www.jacionline.org. If you wish to receive CME or MOC credit for the article, please see the instructions above.

REVIEW

The causes of recurrent infections can be varied, ranging from intrinsic anomalies of the immune system (primary immunodeficiencies) to secondary causes, including viral infections; malignancies; metabolic diseases; congenital anomalies resulting in anatomic defects that might predispose to infections; medications, including immunosuppressive agents, immunomodulatory agents, and cytotoxic drugs; iatrogenic causes; surgery, including removal of immunologically relevant organs (thymus and spleen); and transplantation. Understanding the underlying cause of recurrent infections is important not only for diagnosis but also for appropriate management of the patient. This review focuses on evaluating the humoral (antibody) component of the immune response. The diagnostic laboratory tests used for this purpose are the same whether evaluating a primary or acquired (secondary) defect, although the interpretation, significance, and subsequent management might differ. A review on common variable immunodeficiency (CVID) as part of the Clinical Management series is discussed in an independent article.^{E1}

Overview of assessment of humoral (antibody) immunity

The diagnostic evaluation of a patient with recurrent infections and known or suspected antibody defects, whether primary or secondary, includes at a minimum a detailed clinical history, physical examination, a family history, and laboratory testing. The laboratory component should include quantitative measurement of serum immunoglobulins: IgG, IgA, and IgM (IgE might be included, depending on the clinical history); IgG subclasses (should not be ordered routinely for every evaluation as part of an initial assessment panel, as discussed later in this section); and serologic measurement of antibody responses to vaccine antigens (functional antibody evaluation) (Table E1), which include protein vaccines (DT, tetanus toxoid, and *Haemophilus influenzae* B) and polysaccharide antigens (pneumococcal vaccine and isohemagglutinins). Other vaccines (eg, hepatitis A, hepatitis B, and measles and mumps) can also be used if laboratory testing for these is not readily accessible in a particular region. The measurement of isohemagglutinin levels is not the most robust marker for antibody responses to polysaccharide antigens, especially in very young infants less than 6 months of age, because they are usually detectable in the blood of patients with A, B, or O blood groups only after this age, and therefore it is preferable to assess vaccine responses, when possible. In addition to measurement of immunoglobulin levels and vaccine antibody responses, assessment of humoral immunity should include quantitative B-cell subset immunophenotyping (which is beyond the scope of this article), which, although not useful for diagnostic purposes, has been shown to be relevant and potentially useful in classification and prognosis of patients with CVID.^{E2}

The methodology for measuring serum immunoglobulin levels can vary widely between clinical laboratories; therefore it is essential that laboratory-specific and age-specific reference ranges be used for interpretation and furthermore that serial monitoring of immunoglobulin (IgG) levels for patients receiving replacement therapy is performed in the same laboratory for consistency of data over time. Immunoglobulin levels of the 3 main isotypes, IgG, IgA, and IgM, show distinct age-related variation, and a reliable reference range should include healthy pediatric and adult donors across the age spectrum in each

category. Furthermore, because immunoglobulin levels can vary in pregnant women depending on the stage of pregnancy, it is important to ensure that pregnant women are not included in a general immunoglobulin reference range because this can skew the data and subsequent interpretation of patient results. Most immunoglobulin and IgG subclass reference ranges either use a 95% range (95% CI) or 2 SDs on either side of the mean.

The interpretation of immunoglobulin results should take into account the age of the patient (eg, transient hypogammaglobulinemia of infancy), the clinical history, and other causes that might explain low immunoglobulin levels, including medications and loss of protein in urine or stool. Levels of a single immunoglobulin isotype might be decreased or levels of all 3 major isotypes (panhypogammaglobulinemia) might be decreased, especially in patients with primary humoral or combined immunodeficiencies. Selective IgA deficiency (sIgAD) is the most common immunodeficiency, with a variable incidence reported among various ethnic groups, although it appears to be more prevalent in white and Near Eastern populations than in Far Eastern populations. The majority of patients with sIgAD are asymptomatic, although there can be an increased association with autoimmunity, particularly celiac disease and recurrent infections, in some patients. Laboratory identification of complete IgA deficiency is helpful in selecting the type of immunoglobulin product (IgA depleted) to use for replacement therapy.

Hypogammaglobulinemia is gaining increasing recognition in solid-organ transplant recipients^{E3} and has also been reported in some patients after treatment with B cell-depleting therapy.^{E4} Similar to sIgAD, certain IgG subclass deficiencies (IgG₂, IgG₃, and IgG₄; IgG₁ does not count as a true subclass deficiency because it accounts for the majority of total IgG up to 70%, and therefore a low IgG₁ level would result in low total IgG levels) are also relatively common in the general population (eg, IgG₄ deficiency, which has been reported to be observed in up to 15% of children and 10% of adults, most of whom are otherwise asymptomatic).^{E5} Also, the method used to quantitate IgG subclasses, such as IgG₄, can affect the classification of a deficiency (more sensitive analytic procedures, including ELISA, enable detection of IgG₄ to levels <1 mg/dL). Comparison of patient data must be made with age-matched healthy control subjects to ensure accurate interpretation of IgG subclass results because subclass levels increase with age (especially in the early part of life, 0-2 years). Also, knowledge of testing methodology can determine sensitivity of detection, and the use of local laboratory-derived normal values data is also essential for appropriate interpretation of the data. Other than IgG₄ deficiency, IgG₂ subclass deficiency is the next most common, is often linked to another IgG subclass defect or IgA deficiency, and is also associated with impaired antibody responses to polysaccharide antigens. For these reasons, it has been suggested that IgG subclass assessment be restricted to patients with selective antibody deficiency, sIgAD, or suspected CVID, which is in a relatively early stage of evolution. Thus functional antibody responses to protein and polysaccharide antigens are essential to determining whether an IgG subclass deficiency is clinically relevant and also form the basis of establishing the need for replacement therapy.

It is important to keep in mind that in patients with hypogammaglobulinemia, inability to make specific antibody responses, or both who are being evaluated for fungal or viral infections, alternatives to serologic testing should be used, when possible, to avoid the risk of false-negative results (eg, the Fungitell

[Associates of Cape Cod, East Falmouth, Mass] β -D-glucan assay for invasive fungal infection or the galactomannan antigen test for invasive aspergillosis). For evaluation of viral infections, including HIV, molecular methods, such as PCR, might be more reliable in such patients.

Among the primary immunodeficiency diseases, antibody deficiencies are the most frequent (up to 65%), although antibody/B-cell defects can also be seen in patients with combined immunodeficiencies, as well as other well-characterized primary immunodeficiencies, such as Wiskott-Aldrich syndrome and ataxia telangiectasia. Assessment of the humoral immune compartment includes evaluation of immunoglobulin production, vaccine responses, B-cell immunophenotyping, and other modalities of measuring B-cell function.

Laboratory evaluation of vaccine (functional antibody) responses and interpretation

As previously discussed, the 2 main categories of vaccine responses assessed include protein and polysaccharide vaccines. Because the majority of patients are likely to have received diphtheria and tetanus vaccinations, IgG antibodies to DT and TT are suitable candidates. Frequently, antibodies to *Haemophilus influenzae* B can also be measured for a protein vaccine because it is a conjugate and contains both protein and polysaccharide antigens. It would not be a useful vaccine to measure in countries in which this vaccination is not typically administered. Serologic testing for anti-DT and anti-TT antibodies are widely available. The protective titer is typically determined by the testing laboratory, and a normal or abnormal response is defined by whether the patient has specific antibody titers within or outside the range specified. However, most clinical laboratories have a cutoff of 0.1 IU/mL or greater for these antibodies. Because these antibody titers can wane over time,^{E6} if a vaccination record is not readily available, an option would be to vaccinate the patient and reassess the antibody response 4 to 6 weeks after vaccination. This is particularly true in the adult population, and therefore an initial non-protective titer should not be taken as axiomatic for defective antibody function. Just as with immunoglobulin quantitation, for consistent results and facile interpretation, prevaccination and postvaccination titers should be measured in the same diagnostic laboratory. An ability to mount an effective antibody response (ie, achieve protective titers) would indicate normal humoral immunity to that antigen or group of antigens (protein vaccines). In children the evaluation of vaccine responses should be correlated with the age of the child and the appropriate schedule of vaccination.

Although isohemagglutinins could potentially be assessed in patients receiving immunoglobulin therapy as a surrogate for antibody responses, it has been shown that most immunoglobulin preparations contain varying amounts of isohemagglutinins. In studies evaluating different lots of immunoglobulin preparations for antibodies against blood group antigens, it was shown that anti-A and anti-B antibodies were detected in 88% of these lots and that the reported titers vary from low to high. Therefore this might not be the most suitable approach for measuring antibody responses in patients undergoing immunoglobulin replacement.

Although it might be optimal to evaluate functional antibody responses in a patient not receiving immunoglobulin therapy, this might not always be possible. Because the half-life of IgG is known, it is possible to administer vaccines during

immunoglobulin replacement and measure antibody responses, ensuring that baseline (prevaccine) data, total IgG levels, and postvaccine data are available for interpretation.^{E7,E8} Vaccination of patients with CVID undergoing immunoglobulin replacement has shown that it is possible to assess functional antibody responses in such a context, and in fact, a subset of such patients were demonstrated as being capable of mounting vaccine responses.^{E9}

Neoantigens have been used in some settings to ascertain *de novo* functional antibody responses in patients receiving immunoglobulin therapy. However, the options of neoantigens are fairly limited at present and include the bacteriophage Φ X174, rabies vaccine, and the tick-borne encephalitis virus vaccine.^{E10-E12} The latter is a vaccine used primarily in Europe and therefore not applicable to the North American context. The bacteriophage Φ X174 is available only from a single source at present, with limited access (single-site) to laboratory testing. The bacteriophage is administered at 0 and 6 weeks, and blood for testing is collected at 0, 7, 14, and 28 days after vaccination. An attempt is being made to procure "orphan drug test status" for this neoantigen, which, if successful, might permit wider dissemination and setup of testing facilities.

There are 2 main rabies vaccines available: Imovax (Sanofi Pasteur, Lyon, France) and Rabavert (Chiron Behring GmbH, Marburg, Germany). Although there have been occasional shortages of these vaccines, there is no indication that these vaccines are in short supply at present. The vaccine as a neoantigen is administered at 0 and 12 to 13 weeks. Laboratory testing to measure antibody response to rabies vaccine is available through clinical reference laboratories in at least 3 states in the United States; however, the current methods only measure total titers (not IgG vs IgM). The methods used include either ELISA or a rapid fluorescent focus inhibition test for detection of neutralizing antibodies. The cost of the recommended 3 doses of rabies vaccine administered for exposure or prophylaxis varies depending on accessibility to health insurance. The cost for a patient not covered by insurance can vary depending on location from \$500 to \$1200, whereas for a patient with insurance, the cost can decrease to \$10 to \$40. The rabies vaccine has been shown to be relatively safe and without any significant adverse effects reported relative to the number of vaccine doses administered (<http://www.cdc.gov/vaccines/vac-gen/side-effects.htm>). However, all of these neoantigens are protein antigens and do not provide the means to assess antibody responses to polysaccharide antigens. There is a meningococcal polysaccharide vaccine (Menomune, Sanofi Pasteur) consisting of 4 polysaccharide antigens; however, the key limitation is the lack of widely available and standardized laboratory testing to measure antibody responses to these polysaccharides.

The caveats for use of the protein and polysaccharide neoantigens include accessibility to laboratory testing for the specific antibody and, in the case of Φ X174, access to the bacteriophage preparation for vaccination, as previously indicated. Also, rabies vaccine might not be a suitable neoantigen in countries in which rabies vaccination might be more frequent in the general population.

For polysaccharide antigens, the most common vaccine used is the pneumococcal vaccine against *Streptococcus pneumoniae*. There is a 23-valent vaccine (Pneumovax 23; Merck, Whitehouse Station, NJ) containing all the major serotypes that cause invasive pneumococcal disease and 3 pneumococcal conjugate vaccines (Prenar7 [7-valent], Wyeth, Madison, NJ; Synflorix [10-valent], GlaxoSmithKline, Research Triangle Park, NC; and

Prenar13 [13-valent], Wyeth). The Synflorix vaccine is used in Europe, whereas Prevnar7 has been used in the United States for more than a decade in children less than 2 years of age or children aged 2 to 5 years who have not received prior vaccination and are at high risk for pneumococcal infection. Prevnar13 received US Food and Drug Administration approval 2 years ago, but there is no formal endorsement as yet from the Centers for Disease Control and Prevention on its use in adults older than 50 years.

In some rare cases the meningococcal polysaccharide vaccine can be used; however, interpretation of protective antibody responses is less clearly defined, as alluded to above. Polysaccharide antigen-specific antibody responses are useful not only in patients with humoral primary immunodeficiencies who have hypogammaglobulinemia or agammaglobulinemia but also in patients with normal IgG levels who are unable to respond to such vaccines (impaired polysaccharide responsiveness).

The interpretation of antibody responses to pneumococcal vaccination is challenging and probably somewhat controversial. The clinical laboratories in the United States that offer antibody testing to the 23-valent vaccine (which includes the serotypes represented in the 3 conjugate vaccines) measure IgG antibodies to *S pneumoniae* polysaccharides because it is a type 2 T-independent antigen. It has been recently shown that neutrophils in the marginal zone of the spleen are critical in the development of antibody responses to type 2 T-independent antigens, and in fact, these B-helper neutrophils facilitate isotype class-switching of the antibody response, among other functions. The more widely used laboratory methodology for pneumococcal antibody testing is a fluorescent bead-based quantitative assay, although it is possible to also use a variety of enzyme immunoassay methods for this purpose. Because most of these laboratories perform their own bead conjugation and develop their own standard and calibrators, it is essential to have prevaccination and postvaccine evaluation in the same laboratory.

Practice guidelines and literature recommend the use of a serotype-specific response of 1.3 $\mu\text{g/mL}$ or greater as being indicative of an antibody response to that serotype, and an overall response to the vaccine would include the above cutoff for 50% or more serotypes in children 2 to 5 years of age and 70% or more serotypes in subjects 6 years or older.^{E13} Alternate recommendations suggest using a 4-fold increase in antibody titers over the initial (prevaccination) value as being another measure of the polysaccharide-specific antibody response.^{E13} The 1.3 $\mu\text{g/mL}$ or greater cutoff value is somewhat arbitrary, and because individual serotypes have varying immunogenicity, it is not clear that all serotypes would stimulate an equally effective antibody response; this response might also be unequal depending on age. Furthermore, it is not clear as to what antibody titer constitutes a “response” versus what is protective against infection, which requires longitudinal monitoring after vaccination, especially because antibody titers might not correlate with function in the case of pneumococcal antibodies because protection involves opsonophagocytosis of the bacteria by the serotype-specific antibody (opsonizing capacity) along with complement activation.

At present, there are limited data on pneumococcal antibodies in healthy subjects, either adults or children, who have not been previously vaccinated or have had pneumococcal infection to determine what the antibody responses to the 23 serotypes might be in a healthy population. Also, approximately 30% of the general population might have nasal colonization with *S pneumoniae*, which could affect the magnitude of the response after

vaccination. A recent study in healthy adults who had not been previously vaccinated or had pneumococcal infection included assessment of prevaccination and postvaccination (Pneumovax) IgG antibody responses to all 23 pneumococcal serotypes. Serotype-specific cutoffs were developed for interpretation of “response” to pneumococcal polysaccharides by using the prevaccine and postvaccine antibody data (Park et al, manuscript in preparation)^{E14}; however, the robustness of these cutoffs needs to be prospectively evaluated in a cohort of patients before and after vaccination in a future study. Also, it should be noted that the above-described limitations in interpretation and methodology would unfortunately make such serotype-specific cutoffs and “reference values” laboratory specific, as with other measurements, including IgG subclass levels. Also, such serotype-specific cutoffs before and after 23-valent polysaccharide vaccination have not been developed in children, especially those who have received previous conjugate vaccine.

Recent studies reveal that children less than 2 years of age can indeed produce antibodies to the 23-valent polysaccharide vaccine,^{E15} and more recently, it was shown that the antibodies produced in infants and young children are functional (ie, capable of opsonophagocytic activity).^{E16} An ideal evaluation of the antibody response in patients to pneumococcal polysaccharide vaccination would include serotype-specific antibody quantitation and measurement of antibody function (opsonophagocytic assay).

Future of antibody testing: The next 5 years

The opsonophagocytic assay is very likely to extend beyond research laboratories and enter the clinical diagnostic arena in the next 2 to 3 years, which will facilitate evaluation of functional pneumococcal antibody responses. Additional efforts are required to develop more robust guidelines for interpretation of such antibody responses. Identification of alternate neoantigens and more widespread laboratory testing for measuring neoantigen-specific antibody responses would be helpful in the evaluation of the patient undergoing immunoglobulin replacement in whom traditional protein and polysaccharide antibody measurements cannot be used. Another area that is gaining attention in the diagnostic laboratory is the development of quantitative antibody-secreting assays, whereby antigen-specific antibody responses or polyclonal antibody responses can be assessed by studying antibody-secreting cells (B-cell ELISpot; Millipore, Bedford, Mass). A robust B-cell ELISpot assay might obviate the need for a neoantigen because replacement immunoglobulin therapy would not be expected to interfere with the measurement of antibody-secreting cells. Improvement of laboratory diagnostic methods, including assay standardization and use of reference materials, might permit more meaningful interlaboratory data comparison of immunoglobulin quantitation or functional antibody results.

CASE REVISITED

The clinical, radiologic, and laboratory findings in this patient supported a diagnosis of a primary immunodeficiency, CVID, because secondary causes for the hypogammaglobulinemia were eliminated after careful evaluation. The patient was initiated on replacement immunoglobulin therapy with improvement in the incidence of infections. She was subsequently transferred to subcutaneous immunoglobulin therapy while still maintaining good infection control.

Yearly radiologic examinations revealed resolution of some of the nodules in the lung, particularly after immunoglobulin treatment (Fig E1, B). Periodic evaluation and serial monitoring of immunoglobulin levels to maintain an optimal trough that correlates with and improves clinical outcomes (not just maintenance of a target trough level) are essential for reducing infectious complications of CVID. This patient exemplifies the clinical and laboratory investigations related to antibody testing required to assess patients who present with recurrent infections.

REFERENCES

- E1. Cunningham-Rundles C, Maglione PJ. Common variable immunodeficiency. *J Allergy Clin Immunol* 2012;129:1425-6, e3.
- E2. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. *Blood* 2008;111:77-85.
- E3. Mawhorter S, Yamani MH. Hypogammaglobulinemia and infection risk in solid organ transplant recipients. *Curr Opin Org Transplant* 2008;13:581-5.
- E4. Irie E, Shiota Y, Suzuki C, Tajima Y, Ishizawa K, Kameoka J, et al. Severe hypogammaglobulinemia persisting for 6 years after treatment with rituximab combined chemotherapy due to arrest of B lymphocyte differentiation together with alteration of T lymphocyte homeostasis. *Int J Hematol* 2010;91:501-8.
- E5. Buckley RH. Immunoglobulin G subclass deficiency: fact or fancy? *Curr Allergy Asthma Rep* 2002;2:356-60.
- E6. Amanna JJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med* 2007;357:1903-15.
- E7. Shvarts A, Ballou M, Yu A, Lehman H. An evaluation of pneumococcal titers in patients receiving Ig replacement for immunodeficiency. *J Allergy Clin Immunol* 2012;129:AB84.
- E8. Vasquez A, Rosenblatt HM, Hanson IC, Krance RA, Shearer WT. Evaluation of antibody responses of SCID patients post-bone marrow transplant while receiving IVIg. Abstract presented at: Clinical Immunology Society Meeting; Philadelphia, PA: May 2010.
- E9. Goldacker S, Draeger R, Warnatz K, Huzly D, Salzer U, Thiel J, et al. Active vaccination in patients with common variable immunodeficiency (CVID). *Clin Immunol* 2007;124:294-303.
- E10. Seidel MG, Grohmann E, Sadeghi K, Pollak A, Heitger A, Forster-Waldl E. Vaccination against tick-borne encephalitis virus tests specific IgG production ability in patients under immunoglobulin substitution therapy. *Vaccine* 2010;28:6621-6.
- E11. Brinkman DM, Jol-van der Zijde CM, ten Dam MM, Vossen JM, Osterhaus AD, Kroon FP, et al. Vaccination with rabies to study the humoral and cellular immune response to a T-cell dependent neoantigen in man. *J Clin Immunol* 2003;23:528-38.
- E12. Ochs HD, Buckley RH, Kobayashi RH, Kobayashi AL, Sorensen RU, Douglas SD, et al. Antibody responses to bacteriophage phi X174 in patients with adenosine deaminase deficiency. *Blood* 1992;80:1163-71.
- E13. Sorensen RU, Leiva LE, Javier FC 3rd, Sacerdote DM, Bradford N, Butler B, et al. Influence of age on the response to *Streptococcus pneumoniae* vaccine in patients with recurrent infections and normal immunoglobulin concentrations. *J Allergy Clin Immunol* 1998;102:215-21.
- E14. Park MA, Snyder MR, Smith C, Jenkins S, Ryu E, Therneau T, et al. New guidelines for the interpretation of IgG pneumococcal antibody data: results from a cohort study of healthy adults. *Clin Immunol* 2010;135:38.
- E15. Balloch A, Licciardi PV, Russell FM, Mulholland EK, Tang ML. Infants aged 12 months can mount adequate serotype-specific IgG responses to pneumococcal polysaccharide vaccine. *J Allergy Clin Immunol* 2010;126:395-7.
- E16. Licciardi PV, Balloch A, Russell FM, Burton RL, Lin J, Nahm MH, et al. Pneumococcal polysaccharide vaccine at 12 months of age produces functional immune responses. *J Allergy Clin Immunol* 2012;129:794-800, e2.

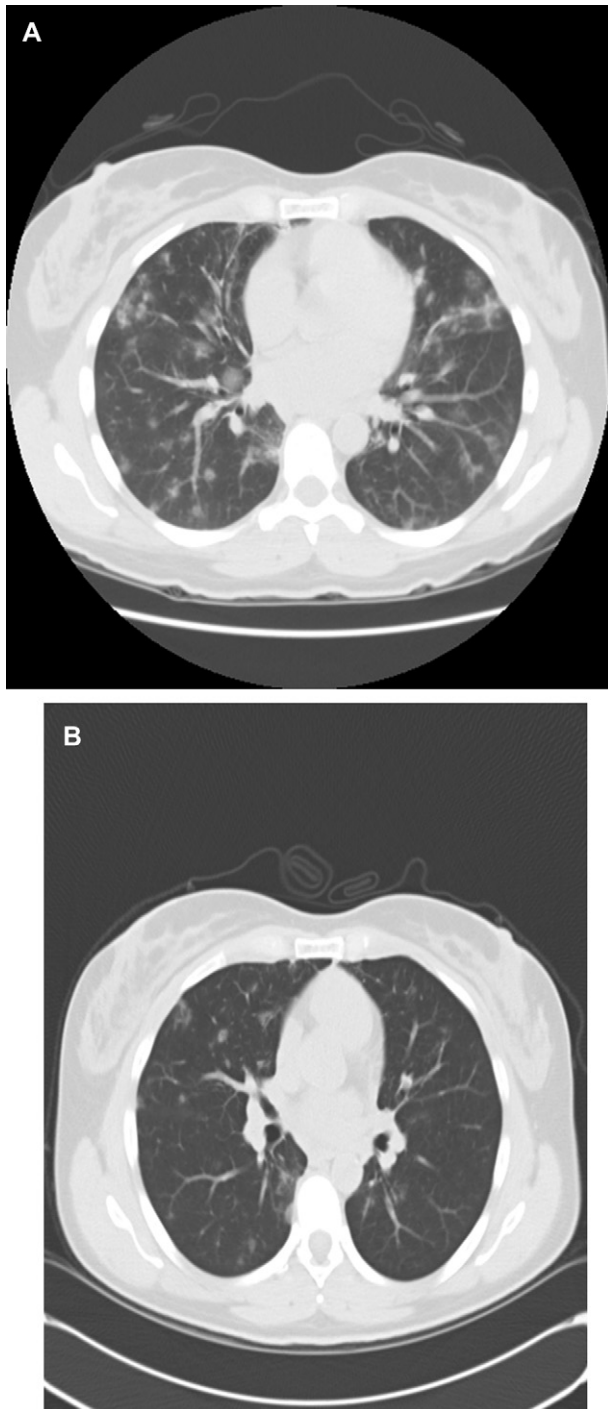


FIG E1. **A**, Computed tomographic scan of the chest reveals mild bilateral hilar lymphadenopathy with ill-defined inflammatory-appearing nodules scattered throughout both lungs. **B**, Computed tomographic scan of the chest reveals extensive bilateral ground-glass density with nodular infiltrates. There is some resolution in the nodules throughout both lungs compared with the image in Fig E1, **A**, which was taken 2 years before this image.

TABLE E1. Diagnostic evaluation of humoral immunity (antibodies) in patients with infections

Quantitative antibody measurements

- a. Serum immunoglobulins (IgG, IgA, and IgM); IgE (useful in recurrent respiratory infections and atopic disease); IgD is not useful for evaluation of antibody deficiencies
- b. IgG subclasses (useful if borderline total IgG or impaired vaccine response, adults and children >1 y)

Functional antibody measurements/vaccine responses

- a. *Isohemagglutinins* (antibodies to polysaccharide antigens, useful only in children >6 mo of age, not useful in AB+ blood group individuals)

b. *Protein antigens*

- i. Diphtheria toxoid (anti-DT)
- ii. Tetanus toxoid (anti-TT)
- iii. *Haemophilus influenzae* B (anti-HiB)
- iv. Other vaccines

c. *Polysaccharide antigens*

- i. *S pneumoniae* polysaccharides
- ii. Meningococcal polysaccharides

d. *Neoantigen*

- i. ϕ X174
- ii. Rabies vaccine
- iii. Others (see text)