

Assessment and clinical interpretation of polysaccharide antibody responses

Kenneth Paris, MD, and Ricardo U. Sorensen, MD

This second article in the miniseries Practical Aspects of Ambulatory Diagnosis and Management of Immunodeficiency Disorders¹ extends the discussion on evaluation of individuals with suspected humoral immunodeficiency² by reviewing the logistics and interpretation of the patient's ability to produce antibodies to polysaccharide antigens, specifically pneumococcal surface polysaccharides. The response to these polysaccharides is important in the evaluation of patients with documented immune abnormalities and those individuals who have normal total immunoglobulin levels. Although profound immune deficiencies, such as X-linked agammaglobulinemia and severe combined immunodeficiency, are always associated with a defect in specific antibody production, some immune disorders may have variable responses, whereas others with persistent IgG or IgG subclass deficiencies may have normal or clearly abnormal antipolysaccharide antibodies. Measurement of the response to pneumococcal polysaccharides is preferred because of the availability of a pure polysaccharide vaccine for antigen challenge and standardized techniques to measure specific antibody responses.

PNEUMOCOCCAL VACCINE RESPONSE

Immunization with a polysaccharide pneumococcal vaccine (eg, Pneumovax 23; Merck, Whitehouse Station, New Jersey; or Pnu-Immune 23; Lederle Laboratories, Madison, New Jersey) is used to evaluate response to polysaccharide antigens in children older than 2 years. Since 2000, use of a conjugate vaccine (eg, Prevnar; Wyeth Labs, Madison) has been standard of care in the United States, and thus, most children born *after* 2000 have been immunized with up to 4 doses of the conjugate vaccine. Those born *before* 2000 have usually not been immunized with either vaccine. In children referred for an immune evaluation, completion of the recommended doses of conjugate vaccine is suggested before immunization with the polysaccharide vaccine after 2 years of age.

Department of Pediatrics, Louisiana State University Health Sciences Center, Children's Hospital of New Orleans, Division of Allergy/Immunology, New Orleans, Louisiana.

Authors have nothing to disclose.

Received for publication June 20, 2007.

Received in revised form July 18, 2007.

Accepted for publication July 23, 2007.

ALTERNATIVE MEASURES OF POLYSACCHARIDE RESPONSE:

Isohemagglutinins are antibodies generated in response to polysaccharides of gut flora that cross-react with A or B blood group erythrocyte antigens.³ However, this has limited utility because most laboratories measure combined titers of IgG and IgM for isohemagglutinins. Although not routinely used in the United States, a polysaccharide vaccine for meningococcus is also available and has been used to measure the polysaccharide response. However, the interpretation of the response to this vaccine is not well established. Also, anti-*Haemophilus influenzae* antibodies do not measure anti-polysaccharide antigen responses, since it is a conjugated vaccine.

VARIABLES THAT AFFECT MEASUREMENT AND INTERPRETATION OF PNEUMOCOCCAL ANTIBODY TITERS

Laboratory standardization rules developed for anti-pneumococcal polysaccharide antibody evaluation include absorption of patient serum samples with polysaccharide C and serotype 22F. We recommend measurement of antibody titers to 12 to 14 different serotypes, ideally including at least 7 serotypes present only in the 23-valent polysaccharide vaccine (Prevnar contains serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) (Table 1). Panels of titers that conform to this recommendation are available through several commercial laboratories.

Titers of IgG antibody concentrations to specific pneumococcal serotypes are determined by a standardized enzyme-linked immunosorbent assay method and are expressed in micrograms per milliliter.⁴ Protection against infection and colonization has been associated with specific IgG concentrations of 1.3 $\mu\text{g/mL}$ or more (or 200 to 300 ng/mL of antibody N in previously used antibody measurements).^{5,6} Lower titers (0.15 $\mu\text{g/mL}$) have been associated with protection from invasive pneumococcal disease (for example, septicemia).

Ideally, evaluation includes measuring titers before and again 3 to 6 weeks after vaccination. It is recommended that the same laboratory perform the prevaccination and postvaccination titers because different laboratories may measure titers to different serotypes. Given the present standardization of the assay, storage of preimmunization and postimmunization blood samples and simultaneous performance of the

Table 1. Pneumococcal Polysaccharide Serotypes Present in the Polysaccharide and Conjugated Pneumococcal Vaccines^a

Serotypes		Vaccines	
Danish No.	US No.	PPV	PCV-7
1	1	X	
2	2	X	
3	3	X	
4	4	X	X
5	5	X	
6B	26	X	X
7F	51	X	
8	8	X	
9N	9	X	
9V	68	X	X
10A	34	X	
11A	43	X	
12F	12	X	
14	14	X	X
15B	54	X	
17F	17	X	
18C	56	X	X
19A	57	X	
19F	19	X	X
20	20	X	
22F	22	X	
23F	23	X	X
33F	70	X	

Abbreviations: PCV-7, polysaccharide conjugate vaccine; PPV, polysaccharide vaccine.

^a Serotypes included in both PPV and PCV-7 are set in boldface type.

assay are no longer necessary (R.U.S., unpublished data, 1995 to present).

INTERPRETING RESPONSES

Unfortunately, no published data are available in healthy individuals that permit a solid definition of normal and abnormal pneumococcal antibody responses in different age groups. Normal responses are highly dependent on patient age and have not been fully defined.⁷ Responses vary considerably among individuals and among serotypes in the same individual. Broader differences in response may also exist with regard to patient ethnicity. We interpret responses to the polysaccharide pneumococcal vaccine based on 3 components.⁴

The first component is increase in specific antibody titers over preimmunization concentrations. An adequate response is 4-fold or higher over baseline for a given serotype. Therefore, a patient with a preimmunization titer of 0.4 $\mu\text{g/mL}$ should attain a postimmunization titer of approximately 1.6 $\mu\text{g/mL}$. Patients with a high preimmunization titer may not generate a 4-fold increase after immunization.⁸

The second component is the final concentration of antibodies after immunization (regardless of increase from preimmunization concentration). An adequate response is a

postimmunization antibody concentration of more than 1.3 $\mu\text{g/mL}$, even in the absence of a 4-fold increase.

The third component is the percentage of serotypes in the vaccine to which the patient responded. Children 2 to 5 years of age are normally expected to have an adequate response to more than 50% of the serotypes tested. If they had previously received conjugate vaccine and have high titers to those serotypes, an adequate response to more than 50% of serotypes *exclusively* found in the polysaccharide vaccine postimmunization is expected. Patients 6 years or older are normally expected to respond to more than 70% of the serotypes tested.

Although these criteria need validation in a range of immunodeficient populations, they were validated as being 73% and 57% sensitive and specific, respectively, in a population of children infected with human immunodeficiency virus for identifying immunodeficient individuals.⁹ In addition, they have helped in the prognostication and treatment of more than 1,000 patients of all ages tested since 1995 (R.U.S., unpublished data, 1995 to present).

Other factors that affect interpretation include (1) previous immunization with pneumococcal vaccines, (2) treatment with intravenous or subcutaneous immunoglobulin within the previous 6 months, and (3) underlying disease states and/or treatments possibly affecting immune response, such as long-term steroid therapy, malignancy, chemotherapy, and others. In those immunized with the conjugated vaccine, pneumococcal antibody titers measured before immunization with the polysaccharide vaccine may represent the patient's response to the conjugate vaccine. Responses to natural infection can also be seen in many patients.

SUBSEQUENT VACCINATIONS AS PART OF EVALUATION OF RESPONSE

Patients with initial poor response to the pneumococcal polysaccharide vaccine may clinically benefit from immunization with the conjugate vaccine.⁴ These vaccines were developed because healthy children younger than 2 years demonstrate poor responses to polysaccharide antigens alone. However, conjugation helps direct the response toward the immunogenic protein complexed to the polysaccharide antigen, thereby stimulating protective immune responses in those individuals. Therefore, the response to Prevnar is *not* used in evaluating polysaccharide antibody responses. Some immunologists recommend giving an additional polysaccharide vaccine after giving the conjugate vaccine. This approach may further increase the response to serotypes included in the conjugate vaccine but is unlikely to increase the response to serotypes found only in the polysaccharide vaccine.

Repeated immunization with the polysaccharide vaccine should not be considered a routine therapeutic option in patients with recurrent infections that failed to respond to the initial immunization. If a patient does not respond to the first dose of the 23-valent vaccine, our experience suggests that a second dose generally has little benefit.⁴ An exception to this may be reimmunization of otherwise immunologically healthy patients whose initial vaccine response resulted in

titers slightly below protective levels (these patients may then have protective titers). Additionally, young patients (2–5 years of age) who initially had a poor response to the polysaccharide vaccine may outgrow their immune deviation. In these cases, this can be monitored by reevaluation (including vaccination with the polysaccharide vaccine) 1 to 2 years after their initial vaccine challenge.

CHARACTERIZATION OF ABNORMAL RESPONSES

Patients with normal immunoglobulin levels may have varying degrees of responsiveness to the polysaccharide vaccine (R.U.S., unpublished data, 1995 to present).¹⁰ Patients with the most severe form of unresponsiveness fail to respond to any serotype. Some of these patients may respond to 1 serotype only. In both cases, if additional immunization with the conjugate vaccine fails to bring a clinical benefit, IgG replacement should be considered. Such consideration should include polysaccharide unresponsive patients who responded serologically to the conjugate vaccine but failed to improve clinically.

Patients with partial responses to some but not all serotypes fall into many categories of clinical severity. Management should include cultures to determine if pneumococci are involved in causing infection, additional immunization with the conjugate vaccine, and aggressive antibiotic treatment. IgG replacement is rarely indicated.

In cases of clinical deterioration (ie, worsening of infections or increasing frequency of infections) 6 to 12 months after initial immunization, reevaluation of antibody titers may demonstrate rapid loss of protective titers. The normal rate of decrease of antibody concentrations after immunization has not been defined, but a correlation between increasing infection frequency and loss of protective titers suggests a causal relationship. Patients who initially showed improvement in antipneumococcal IgG titers after pneumococcal polysaccharide vaccine immunization and who subsequently lost protective titers usually respond by increasing titers after reimmunization with the polysaccharide vaccine. In the absence of clinical deterioration, routine reassessment of antibody titers is not necessary.

CONCLUSION

Clinical disease staging and treatment of patients referred for recurrent infections need to be considered within the context of the types of infections that affect the patient, the results of initial immunologic screening tests, and the patient's ability

to produce specific antibodies. The varied approach to treatment of these complex patients is beyond the scope of this review. A brief discussion of IgG replacement and treatment strategies is found in the article by Agarwal and Cunningham-Rundles.² Patients with normal total immunoglobulin levels and abnormal specific antibody production should also be considered for IgG replacement if other treatment modalities (immunization with the conjugate vaccine, prophylactic antibiotics) fail to result in decreases in infection frequency.

REFERENCES

1. Dinakar C. Practical aspects of ambulatory diagnoses and management of immunodeficiency disorders. *Ann Allergy Asthma Immunol.* 2007;99:201–202.
2. Agarwal S, Cunningham-Rundles C. Assessment and clinical interpretation of reduced IgG values. *Ann Allergy Asthma Immunol.* 2007;99:281–283.
3. Fong SW, Qaundah BY, Taylor WF. Developmental patterns of ABO isoagglutinins in normal children correlated with the effects of age, sex, and maternal isoagglutinins. *Transfusion.* 1974;14:551–559.
4. Sorensen RU, Leiva LE, Giangrosso PA, et al. Response to a heptavalent conjugate *Streptococcus pneumoniae* vaccine in children with recurrent infections who are unresponsive to the polysaccharide vaccine. *Pediatr Infect Dis J.* 1998;17:685–691.
5. Landesman SH, Schiffman G. Assessment of the antibody response to pneumococcal vaccine in high-risk populations. *Rev Infect Dis.* 1981;3(suppl):S184–S197.
6. Lawrence EM, Edwards KM, Schiffmann G, Thompson JM, Vaughn WK, Wright PF. Pneumococcal vaccine in normal children: primary and secondary vaccination. *Am J Dis Child.* 1983;137:846–850.
7. Bonilla FA, Bernstein IL, Khan DA, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *Ann Allergy Asthma Immunol.* 2005;94(suppl):S1–S63.
8. Sorensen, RU, Leiva, LE, Javier FC III, 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;102:215–221.
9. Kamchaisatian W, Wanwatsuntikul W, Sleasman JW, Tangsinmankong N. Validation of current joint American Academy of Allergy, Asthma & Immunology and American College of Allergy, Asthma and Immunology guidelines for antibody response to the 23-valent pneumococcal vaccine using a population of HIV-infected children. *J Allergy Clin Immunol.* 2006;118:1336–1341.
10. Go ES, Ballas ZK. Anti-pneumococcal antibody response in normal subjects: a meta-analysis. *J Allergy Clin Immunol.* 1996;98:205–215.