

### Interpretation of post-pneumococcal vaccine antibody levels: Concerns and pitfalls

Caitlin M.G. McNulty, MD, and James T. Li, MD



#### Clinical Implications

- In the workup of immunodeficiency, PPSV23 vaccine challenge and interpretation should be approached with caution and reserved for cases with a high index of suspicion.

#### TO THE EDITOR:

Response to the polyvalent pneumococcal immunization PPSV23 has been used for the past 3 decades to measure the immune response to polysaccharide antigens. The bacterium *Streptococcus pneumoniae* is covered with a cell wall containing cell wall polysaccharide and is further encased by a capsule consisting of repeating polysaccharide epitopes. These epitopes are unique to individual pneumococcal serotypes and play a key role in directing the immune response against this microorganism. Measurement of antibody to these serotype-specific epitopes may be used to assess the immune system's response to polysaccharide antigen.

Historically, radioimmunoassay (RIA), which measures IgG, IgA, and IgM, was used to demonstrate antibody response; this technique has been replaced by ELISA and bead-based multiplexed assays. Opsonophagocytic assays (OPAs) have also been used to measure functional antibody; however, the labor-intensive nature of this testing limits widespread implementation.<sup>1</sup> The 2015 guidelines for primary immunodeficiency (PID) that discuss interpretation of postvaccination antibody levels are predicated on the World Health Organization ELISA protocol using reference samples from healthy, vaccinated volunteers.<sup>2</sup> Bead-based assays, however, are developed and calibrated by individual laboratories. Therefore, initial and postimmunization quantitative levels of antibodies should be run by the same laboratory.

It is important to note that studies performed before 2000 did not perform 2 necessary reabsorption steps, now considered standard for nonspecific antibody. Nevertheless, these older studies are still cited in support of current guidelines. Preabsorption for cell wall polysaccharide and 22F is considered standard in third-generation ELISA and improve correlation with OPAs.<sup>1</sup> Studies performed before this overestimate the protective threshold or fold increases and may not be reliable in determining clinically important pneumococcal vaccine responses.

According to the most recent practice parameter for PID, a normal response to PPSV23 can be defined as achievement of a specific numeric threshold representative of protection or as a 2- to 4-fold increase in quantitative levels of antibody.<sup>2</sup> According to this source, a 2- or 4-fold increase in 50% of tested serotypes represents a normal response in children 5 years or younger; in adults, 70% of serotypes must achieve a 2-

4-fold response to be deemed normal. We question the strength of evidence supporting these recommendations.

The present proposed threshold of greater than or equal to 1.3  $\mu\text{g/mL}$  was derived from studies using RIA technology; a conversion factor of 160 ng N/mL per 1  $\mu\text{g/mL}$  of specific serotype antibody allowed this value to be used for ELISAs.<sup>2</sup> Importantly, these values measured by RIA and non-preabsorbed ELISAs detected nonprotective antibodies; this overestimates the protective threshold and may raise concern for inadequate response when in fact the patient demonstrates protective immunity. Furthermore, different pneumococcal serotypes have varying immunogenicity and serotypes require different levels of antibody for effective opsonization.<sup>1</sup> Therefore, using a single threshold to represent protection may not be a clinically valid way of defining a significant response.

Greater than or equal to 0.35  $\mu\text{g/mL}$  is accepted as the protective threshold against invasive pneumococcal disease according to a meta-analysis of 3 studies of infants receiving PCV13 using ELISAs with both preabsorption steps.<sup>3</sup> When using the cutoff of greater than or equal to 1.3  $\mu\text{g/mL}$  for 12 pneumococcal serotypes, 75% of patients receiving immunoglobulin replacement had deficient antibodies to pneumococcal serotypes despite therapeutic IgG trough values and protective levels against *Haemophilus* in 93% of patients.<sup>4</sup> However, when using the threshold of greater than or equal to 0.35  $\mu\text{g/mL}$ , only 20% of these patients had deficient antibodies.<sup>4</sup> This may suggest that greater than or equal to 0.35  $\mu\text{g/mL}$  is more representative of protection than the greater than or equal to 1.3  $\mu\text{g/mL}$  threshold value.

A 2- to 4-fold increase from baseline has conventionally been used to define a significant pneumococcal antibody response. One retrospective analysis of healthy patients and patients subsequently diagnosed with PID found that a 2-fold response to vaccination was consistent across the 14 serotypes assessed; this article was not designed to correlate phenotype with fold increase.<sup>5</sup> Therefore, 2-fold increases may lack the sensitivity to distinguish between healthy patients and patients with PID.<sup>5</sup> A 4-fold increase in postvaccination quantitative levels of antibodies had a sensitivity of only 73% and a specificity of only 57% in distinguishing immunocompetent patients from patients with HIV.<sup>6</sup> Moreover, the generalizability of the 4-fold increase to PID is unclear and may be inappropriate. Furthermore, preimmunization quantitative levels of antibodies can influence fold increase and patients with high preimmunization quantitative levels of antibodies are less likely to mount a 4-fold response.<sup>5,6</sup>

The percentage of antibodies to pneumococcal serotypes that achieve a "significant" response, as defined by either fold increase above baseline or achievement of a specific threshold, has been proposed as a way to determine whether the overall vaccine response can be considered normal; the quality of this evidence is poor.<sup>7</sup> In the study noted previously, a greater than or equal to 4-fold increase above baseline for 6 of the 12 serotypes assessed was 73% sensitive and 57% specific in distinguishing patients with HIV.<sup>6</sup> The response to polysaccharide antigens increases with age in pediatric patients in what Hidalgo et al termed a "step-wise,

age-dependent manner” beyond the age of 2 years.<sup>8</sup> By age 5 years, this response has matured.<sup>8</sup> The 70% cutoff that has been proposed for older children and adults was established by a consensus group in the 2005 practice parameter on the diagnosis of PID.<sup>9</sup>

Specific antibody deficiency (SAD) is defined as an insufficient response to pneumococcal vaccination despite normal protein antigen response in the setting of recurrent respiratory infections. The diagnostic criteria established by the 2015 PID practice parameter are based on expert opinion and use of ELISA for quantitative levels of antibody assessment.<sup>2</sup> Because of reliance on outdated technology, because of weak evidence supporting a greater than or equal to 1.3 µg/mL threshold, and because of weak evidence supporting a 2- or 4-fold increase in antibody, a very high index of suspicion for SAD is warranted to avoid overdiagnosis.

Most compelling are studies clearly showing that conventionally used criteria to diagnose impaired pneumococcal antibody responses fail to distinguish healthy controls from immunodeficient patients. A recent study of the application of the updated 2015 PID criteria classified more than 40% of healthy students as exhibiting impaired pneumococcal responses.<sup>10</sup> Applying any of the conventionally used criteria for impaired pneumococcal antibody responses in the wrong clinical context risks overdiagnosis.

PPSV23 can be used in the diagnosis of humoral immunodeficiencies. However, the utility of antipneumococcal antibody quantitative levels in the evaluation of immunodeficiency suffers from lack of clear evidence-based criteria defining a normal response. The greater than or equal to 1.3 µg/mL threshold likely significantly overestimates the protective threshold, whereas use of fold increases suffers from lack of consensus regarding what magnitude increase is clinically significant. Using a cutoff value of greater than or equal to .35 µg/mL may be more reflective of protection. OPAs may be more accurate and clinically relevant than the ELISA or bead-based assays. Furthermore, pneumococcal antibody levels are influenced by the performing laboratory and methodology, patient age, serotype immunogenicity, prevaccine quantitative levels of antibodies, and vaccination history. The percentages of serotypes achieving a significant response, used to separate healthy patients from patients with impaired responses, are largely based on outdated technology, retrospective studies, and comparison groups with acquired immunodeficiency. Not surprisingly, the commonly used thresholds defining normal and impaired pneumococcal antibody responses fail to reliably distinguish immunodeficient

patients from healthy controls. For these reasons, use of PPSV23 as a diagnostic tool in immunodeficiency should be limited to cases with a high index of suspicion for PID.

Mayo Clinic Division of Allergic Diseases, Rochester, Minn

Conflicts of interest: J. Li consults for GlaxoSmithKline plc. C. McNulty declares no relevant conflicts of interest.

Received for publication May 14, 2018; revised December 13, 2018; accepted for publication December 14, 2018.

Available online December 27, 2018.

Corresponding author: Caitlin M.G. McNulty, MD, Mayo Clinic Division of Allergic Diseases, 200 First St SW, Rochester, MN 55905. E-mail: [cmcnulty@stpaulallergy.com](mailto:cmcnulty@stpaulallergy.com).

2213-2198

© 2019 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaip.2018.12.016>

## REFERENCES

1. Concepcion NF, Frasch CE. Pneumococcal type 22F polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol* 2001;8:266-72.
2. Bonilla FA, Khan DA, Ballas ZK, Chinen J, Frank MM, Hsu JT, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *J Allergy Clin Immunol* 2015;136:1186-1205.e77.
3. Siber GR, Chang I, Baker S, Fernsten P, O'Brien KL, Santosham M, et al. Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. *Vaccine* 2007;25:3816-26.
4. Jolles S, Jones R, Ponsford MJ, Moody M, Selwood C, El-Shanawany T. Analysis of specific antibody levels to Tetanus, HiB, and pneumococcus in patients with antibody deficiency receiving immunoglobulin replacement. *J Allergy Clin Immunol* 2015;135:AB96.
5. Hare ND, Smith BJ, Ballas ZK. Antibody response to pneumococcal vaccination as a function of preimmunization quantitative levels of antibody. *J Allergy Clin Immunol* 2009;123:195-200.
6. 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;118:1336-41.
7. Orange JS, Ballou M, Stiehm ER, Ballas ZK, Chinen J, De La Morena M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: a working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol* 2012;130:S1-24.
8. Hidalgo H, Moore C, Leiva LE, Sorensen RU. Preimmunization and post-immunization pneumococcal antibody quantitative levels of antibodies in children with recurrent infections. *Ann Allergy Asthma Immunol* 1996;76:341-6.
9. Bonilla FA, Bernstein IL, Khan DA, Ballas ZK, Chinen J, Frank MM, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *Ann Allergy Asthma Immunol* 2005;94:S1-63.
10. Hoffman TW, Van Kessel DA, Rijkers GT. Impact of using different response criteria for pneumococcal polysaccharide vaccination for assessment of humoral immune status. *J Clin Immunol* 2018;38:149-52.