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Reference Laboratory Agreement on Multianalyte Pneumococcal Antibody Results: An Absolute Must!

Harry R. Hill,[a,b] Jerry W. Pickering[b]

Departments of Pathology, Pediatrics, and Medicine, University of Utah, Salt Lake City, Utah, USA[a]; ARUP Laboratories, Salt Lake City, Utah, USA[b]

In this issue, Zhang et al. of the Cleveland Clinic report on an interesting, and what we consider a critical, analysis of multianalyte pneumococcal antibody testing at three major reference laboratories in the United States (1). The determination of the immune response to pure polysaccharide, or conjugated polysaccharide, pneumococcal vaccination has become a critical part of the workup of patients suspected of having the whole spectrum of antibody deficiency diseases. These include patients with the most common immunodeficiency, common variable immunodeficiency, as well as individuals with less common but often quite serious IgG subclass deficiency (particularly, IgG2 deficiency), as well as what is known as "specific polysaccharide antibody deficiency." These individuals fail to make antibody to pure polysaccharides even though their overall IgG, IgG subclasses, and responses to protein vaccines may be perfectly normal.

The initial testing that led to licensing of the pure pneumococcal polysaccharide vaccine, as well as the initial Prevnar7 conjugated pneumococcal vaccine, was carried out mainly by employing enzyme-linked immunosorbent assay (ELISA) methodology to determine immune responses (2, 3). We at ARUP Laboratories had a great deal of experience with the CDC-approved and FDA-standardized ELISAs (2, 3) for measuring pneumococcal antibodies, as we had a 5-year contract to study pre- and postvaccination responses to the Prevnar7 vaccine in follow-up studies after licensing and approval of the vaccine by the FDA. Needless to say, we found this ELISA to be very tedious and fraught with a number of difficulties. For this reason and because we were the first reference laboratory, we believe, to begin employing the Luminex multianalyte bead assay, we devised a 14-valent pneumococcal antibody panel to measure pre- and postvaccination responses and correlated it directly with the standard CDC, FDA, and Wyeth Lederle ELISA methodology. The Luminex methodology offered several significant advantages, including the requirement for a much smaller volume of serum to test multiple analytes and a much larger dynamic range, as pointed out in our initial paper (4) on this methodology and also noted by Zhang et al. in this issue (1). Our work, which is cited as reference 14 by Zhang et al., was the first to investigate by using a multianalyte system to detect pneumococcal antibodies. We definitely felt it was superior to the ELISA system, which was at that time the gold standard, and some of the Wyeth Lederle investigators were even included as authors of the original publication in the *American Journal of Clinical Pathology* (4). For the reasons noted above, almost all reference laboratories have now migrated away from the tedious ELISA format, which requires a significantly larger volume of serum, to various multianalyte systems.

Zhang et al. found a very good agreement of over 80% among three reference laboratories in the United States for protective concentrations of antibody in the sera of 57 mostly adult patients (1). This is assuming that levels of approximately 1.3 μg/ml are protective in adult individuals. The commercially available multianalyte assay marketed by Luminex and used at the Cleveland Clinic gave reasonable results and compared well with laboratory A. In most cases, laboratory B had somewhat lower antibody concentrations. In reviewing that article, I immediately suspected that the laboratory with the lower value must have been our laboratory, as Jerry Pickering and other coworkers, including myself, have reported on a method to eliminate false-positive results in our multianalyte pneumococcal antibody assay by using bovine serum albumin-free StabliGuard immunoassay stabilizer (SurModics, Inc., Eden Prairie, MN) This component allowed us to use more concentrated serum dilutions for the assay and greatly improved the resolution of the assay at the lower end of the curve without incurring higher background values. When I suggested to the section editor that ARUP Laboratories must have been the laboratory with the lower results, I was informed, however, that this was not the case. Thus, other explanations for laboratory B's lower value must be sought. Even with lower values, however, the agreement among the three labs about which sera had protective concentrations of type-specific antibody remained quite good at over 80%.

As pointed out and noted above, the Cleveland Clinic study examined 57 mostly adult patients by using single serum samples (1). I personally see both adult and pediatric patients with immunodeficiency disorders, including possible antibody deficiency, and almost always obtain both prevaccination and 1-month postvaccination serum samples for antibody determination. This, in my opinion, is the only way to determine the adequacy of the postvaccination response. Clearly, we all should work toward performing the same type of study with both pre- and postvaccination samples, as these data would be of more value to clinicians attempting to document antibody deficiency in patients at each of the three participating laboratories, as well as in other laboratories.

Recently, Schutz and colleagues from the Hanover Medical School, the University of Rome—La Sapienza, and several other European universities, along with the Binding Site, have reported on the kinetics of IgM and IgA antibody responses to 23-valent pneumococcal polysaccharide vaccination and have shown that

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Address correspondence to Harry R. Hill, Harry.Hill@path.utah.edu.

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these responses peak at 2 to 3 weeks and remain at a plateau for as long as 3 months (5). Both isotypes of these antibodies remained elevated for as long as 1 year following immunization. The authors speculate that these IgM and IgA responses could be used to determine vaccine responses in people already on immunoglobulin replacement therapy. This is a significant problem, as patients often get placed on immunoglobulin replacement therapy, which consists of greater than 98% IgG, prior to having adequate documentation of an inability to make a specific antibody. Being able to test for IgA and IgM production would be an added benefit to determine the responses to pneumococcal polysaccharides in patients already receiving intravenous or subcutaneous IgG. The problem is complicated, however, by the high concentrations of pneumococcal antibodies in almost all IgG preparations, which could block adequate exposure to vaccination, much like the low doses of IgG originally incorporated into the measles vaccine prevented vaccine-induced rash but also blocked adequate long-term measles immunity. I doubt that testing for pneumococcal IgM or IgA will gain widespread use in the United States for this reason.

Lastly, I congratulate the group from the Cleveland Clinic for carrying out this important study, published in this issue (1), and urge them to continue the exploration of the agreement of such testing among the various reference laboratories. These are critical studies, in my opinion, which should aid in the diagnosis and management of antibody-deficient patients who suffer serious, life-threatening infections due to pneumococci and other polysaccharide-coated bacterial pathogens throughout the United States and the rest of the world.

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