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A NEW 23-SEROTYPE PNEUMOCOCCAL IGG IMMUNOASSAY TO SUPPORT DIAGNOSTIC VACCINATION

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DIAGNOSTIC VACCINATION AS AN IMMUNOLOGICAL INVESTIGATION

Patients with inborn errors of immunity (IEI) present clinically with infectious diseases and disorders of immune regulation. Most IEI patients have impaired antibody-mediated immunity, which may be quantitative, qualitative or both. Assessing humoral immunity is, therefore, a critical component of the IEI workup.[1] Antibody deficiencies cause recurrent respiratory tract and gastrointestinal infections, and the first steps towards investigation are quantitative assessment of serum immunoglobulins (IgA, IgM, IgG, IgE) and lymphocyte subpopulations, and diagnostic vaccination.

Impaired production of antigen-specific antibodies, poor-quality antibodies or poor B cell memory formation is seen in many IEIs and secondary immunodeficiencies. A crucial diagnostic tool for defects in antigen-specific antibody production is the measurement of specific antibodies before and after antigenic stimulation with a vaccine. Defects in B lymphocyte development, maturation, and interactions with T lymphocytes can all be associated with deficient specific antibody production.[2] Testing of vaccine responses form part of diagnostic criteria for conditions like transient hypogammaglobulinemia of infancy (THI) and common IEIs like common variable immunodeficiency, IgG subclass deficiency, selective IgA deficiency and specific antibody deficiency (SAD).[1, 2] Patients with SAD have frequent otosinopulmonary infections caused by encapsulated bacteria, normal serum levels of immunoglobulins and immunoglobulin subclasses, a normal specific antibody response to protein and conjugate vaccines, and an impaired response to pure polysaccharide vaccine.[2]

FDA-approved vaccines indicated for the prevention of communicable diseases are ideal for use in diagnostic vaccination since they provide standardised vaccine antigens at standard dosages with stringently regulated adjuvant content and routes of administration. Diagnostic vaccination is, therefore, relatively standardised across populations. Since vaccine responses are influenced by age, age-appropriate response limits should be used for interpretation.[1] It is important to note that vaccine responses may be affected by transient illness or medication (corticosteroids, anti-epileptics, biologics, immunosuppressants, etc.), and unexpected results should always be confirmed with a repeat test. Diagnostic vaccination should be performed with both a protein and a pure polysaccharide vaccine.[1]

PROTEIN AND POLYSACCHARIDE VACCINES

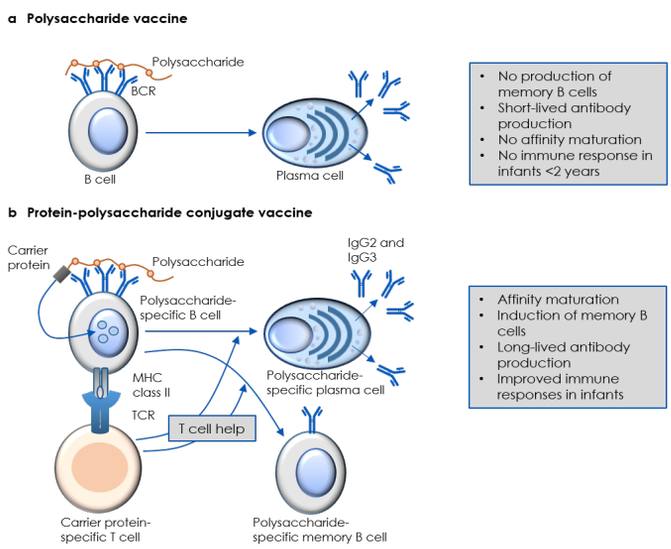
Commonly used protein vaccines include tetanus and diphtheria toxoids. Protein antigens are digested and presented as peptides to T cell receptors (TCR) on CD4+ T helper cells.3,4 Activated CD4+ T cells then provide the activation signal for B lymphocytes to class-switch and produce antigen-specific antibodies. Protein vaccines therefore stimulate T cell-dependent antibody responses, and normal T cell function, in addition to normal B cell function, is required to make it work. Conjugate vaccines like pneumococcal conjugate vaccine (e.g. Prevenar 13) consist of polysaccharide antigens bound to a carrier protein to make them more immunogenic. Prevenar 13 is therefore not a pure polysaccharide vaccine. Similar to protein vaccines, conjugated vaccines stimulate T cell-B cell cooperation.[3, 4] T cell-dependent antibody responses generate robust immunity with strong T cell memory formation, are long-lived, and can be induced in small children with immature immune systems, and patients non-responsive to pure polysaccharide antigens.

Pure polysaccharide vaccines like polyvalent pneumococcal vaccine (Pneumovax 23) stimulate T cell-independent antibody resposes because the pure polysaccharide antigens interact directly with B cell receptors (BCR). The response to pure polysaccharide antigens is therefore a true reflection of B cell function.[3,4] However, pure polysaccharide antigens are naturally less immunogenic and are poorly memory-forming. The pure polysaccharide response is underdeveloped in young children ≤ 2-3 years of age and can only be reliably assessed in older children.

The pure polysaccharide response is naturally short-lived, and waning occurs within 2-5 years in the absence of exposure. It is therefore not unusual for healthy individuals to have low levels of pneumococcal serotype-specific antibodies at random testing, and the clinical value of a random test is therefore extremely limited. An evaluation of the pure polysaccharide response should always include a diagnostic vaccination, even if baseline pneumococcal antibodies appear adequate.

The mechanism of action of polysaccharide and protein/conjugate vaccines is illustrated in Figure 1.

FIGURE 1: MECHANISMS OF IMMUNE ACTIVATION BY PROTEIN, CONJUGATE AND POLYSACCHARIDE VACCINES



Adapted from Pollard AJ et al. Nature Reviews Immunology 2021;21: 83-100.

PNEUMOCOCCAL VACCINE SEROTYPES AND SEROTYPE-SPECIFIC ANTIBODIES

Pneumococcal vaccines are recommended for the prevention of invasive pneumococcal disease (IPD) in childhood as part of the Expanded Programme on Immunisation in South Africa (EPI-SA), adults older than 65 years, and certain high-risk groups.[5] Pneumococcal nasopharyngeal carriage precedes IPD, with colonisation rates highest in children ≤5 years.[6,7] Colonisation rates decrease with age in healthy children and after pneumococcal vaccination.

Pneumococcal vaccination induces the production of serotype-specific IgG antibodies, which peak after about 4-6 weeks and wane within 2-5 years. Pneumococcal vaccines target 23 disease-causing serotypes. Serotype-specific IgG levels associated with protection against IPD vary between serotypes, but protective levels are generally considered to be ≥1.3 ug/ml in adults and ≥0.35 ug/ml in children ≤ 2 years of age.

Levels >0.35 ug/ml have been shown to protect against IPD, but higher serum levels are generally required to prevent nasopharyngeal carriage and localised mucosal infections (sinusitis, otitis, bronchitis, pneumonia).[1, 8-10] Adequate antigen-specific IgG levels in serum do not always translate into adequate protection at mucosal surfaces, where secretory IgA or IgG produced by mucosal B cells may be more important.[7] The 0.35 ug/ml cut-off associated with protection is relevant only to IPD prevention, and not to other endpoints such as acute otitis media, uncomplicated pneumonia or nasopharyngeal carriage.[9] Cut-offs that prevent nasopharyngeal carriage are serotype-dependent and could range from 0.50-2.54 ug/ml.[7]

The prevalence of disease-causing vaccine serotypes in South Africa (SA) has decreased since 2009 following the introduction of childhood pneumococcal conjugate vaccination (PCV). However, the prevalence of non-vaccine serotypes has increased and now dominates in pneumococcal isolates.[6-11] About half are resistant to penicillin and erythromycin. The most common disease-causing serotypes in the post-PCV era include serotype 8 (18%), 12F (6%), 15B/C (5%), 16F (5%) and 19F (8%).[6] Other commonly detected serotypes are 3, 4, 6C, 11A, 19A, 21, 22F, 23A/B, 35B/35D, and 35F.[6,11,12] Circulating serotypes undergo natural fluctuations as well as lineage expansion and lineage replacement, and molecular surveillance is essential to monitor disease-causing serotypes.

INTERPRETING THE ANTIBODY RESPONSE AFTER VACCINATION WITH PNEUMOVAX 23

The 23-serotype polyvalent pneumococcal vaccine (Pneumovax 23) is the recommended polysaccharide vaccine for diagnostic vaccination of adults and children ≥ 2-3 years with suspected primary or secondary immunodeficiency.[1] Vaccine testing detects B cell dysfunction with 55% sensitivity and 84% specificity.[2]

Pneumovax 23 contains capsular polysaccharide antigens of 23 serotypes, 13 of which are shared with Prevenar 13, and 11 of which are targeted only by Pneumovax 23 (pure polysaccharide serotypes). Conjugate vaccination induces potent, long-lasting T cell immunity to the 13 shared serotypes. Vaccination with Pneumovax 23 in patients who received Prevenar 13 elicits both a T cell memory response against shared serotypes and a pure polysaccharide response against pure polysaccharide serotypes. Vaccination and sample collection must be timed correctly, with blood collection at baseline, peak (4-6 weeks) and after 6 months (memory).

A post-vaccination level of ≥1.3 µg/ml plus a 2-fold increase should be observed in at least 70% of serotypes in patients older than 6 years. In children aged 2-6 years, a post-vaccination level of ≥1.3 µg/ml plus a 2-fold increase should be observed in at least 50% of serotypes. Levels ≥1.3 µg/ml should be maintained for at least 6 months.

An inadequate response to Pneumovax 23 vaccination suggests impaired B cell function, and further investigation is recommended. Diagnostic vaccination with a protein vaccine like tetanus toxoid should also be performed to assess T cell-dependent antibody production. Secondary causes of suppressed vaccine responses or rapid waning should always be excluded first. The severity of the impairment can be further classified by determining a patient's response phenotype as per Table 1. It is important to keep in mind that previous Prevenar 13 vaccination confounds interpretation of the polysaccharide response after Pneumovax 23 in some patients, due to shared serotypes that stimulate T cell function.[13] If SAD is still suspected clinically in a patient with an apparently normal response to Pneumovax 23, the response to the 11 pure polysaccharide serotypes (*) should be assessed separately. The pure polysaccharide serotypes are 2*, 8*, 9N*, 10A*, 11A*, 12F*, 15B*, 17F*, 20*, 22F* and 33F*.[2,13] Patients with SAD respond normally to protein and conjugate vaccines, but not to pure polysaccharide antigens (primary polysaccharide non-responsiveness).

Serotype-specific IgG levels vary widely in healthy subjects over time due to natural infection, differences in antigenicity, and natural waning. Finding low antibody levels to some serotypes but not to others is not necessarily indicative of immunodeficiency. Natural waning in vaccinated healthy subjects produces low antibody levels to many serotypes within 2-5 years in the absence of intercurrent exposure.[3,4] An immunological evaluation should therefore always include diagnostic vaccination with protein and polysaccharide vaccines, with a 6-month post-vaccination assessment of B cell memory formation. Waning is considered abnormal if antibody levels decrease to <1.3 ug/ml within 6 months after Pneumovax 23 vaccination in >50% of serotypes in children <6 years, or >70% of serotypes in patients >6 years. An IEI may be diagnosed after careful consideration of the results within the clinical context of the patient and in conjunction with microbiological and other immunological investigations.

It is important to keep in mind that repeated doses of Pneumovax 23 within a short time frame (<5 years) may cause paradoxical inhibition of polysaccharide responsiveness that may confound the clinical interpretation. Pneumovax 23 vaccine should therefore be used judiciously, only for immunological evaluation, and only when immunodeficiency is strongly suspected.

TABLE 1: PNEUMOCOCCAL VACCINE RESPONSE PHENOTYPES

Phenotype	Age >6 years	Age 2-6 years
Severe	≤ 2 protective serotypes	≤ 2 protective serotypes
Moderate	<70% protective serotypes	<50% protective serotypes
Mild	<2-fold increase above 1.3 ug/ml in 70% of serotypes	<2-fold increase above 1.3 ug/ml in 50% of serotypes
Memory	Loss of response within 6 months	Loss of response within 6 months

Adapted from Orange JS et al. J Allergy Clin Immunol (September 2012)

Polysaccharide responsiveness is not routinely assessed in children < 2-3 years of age due to immaturity of the polysaccharide response.[1] These children are also still receiving their primary conjugate vaccine series. Pneumococcal antibodies can be measured if an IEI is suspected in a vaccinated child with frequent infections, but should be interpreted according to the age-appropriate expected response. A 2-fold increase in ≥50% of serotypes should occur following vaccination with Prevenar 13 in healthy infants and small children. Levels <0.35 ug/ml in ≥50% of serotypes in a vaccinated child should prompt an IEI work-up.

WHEN TO CONSIDER A DIAGNOSTIC VACCINATION

Diagnostic vaccination should be considered in patients with:

- Recurrent infections (e.g. sinusitis, otitis media, pneumonia)
- A history of invasive pneumococcal disease
- If a primary immunodeficiency is strongly suspected on clinical grounds
- Risk factors for secondary immunodeficiency (e.g. immunosuppressive therapy, chronic illness, diabetes mellitus, post-splenectomy, etc.).

CLINICAL INTEGRATION AND NEXT STEPS

An inadequate response to protein and/or polysaccharide vaccines should be investigated further. The following steps may be considered:

- **Comprehensive Immune Evaluation:** Assess total immunoglobulin levels (IgG, IgA, IgM, IgE), lymphocyte subpopulations and memory B cells.

- **Management:**
  Antibiotic prophylaxis with/without Immunoglobulin replacement therapy to control frequent infections.

- **Specialist referral:**
  Clinical assessment for complications and end-organ damage (e.g. chronic suppurative otitis media, bronchiectasis, chronic liver disease, enteropathy, autoimmune disease, and splenomegaly).

KEY TAKEAWAYS

- Diagnostic vaccination to assess B cell function should always include vaccination with protein and polysaccharide vaccines.
- The Pneumovax 23 vaccine test, with measurement of pneumococcal IgG antibodies, plays a crucial role in identifying immune deficiencies, particularly in patients with recurrent infections.
- The 23-serotype assay is more accurate and reliable than the 13-serotype assay because it includes pure polysaccharide serotypes that are only targeted by Pneumovax 23.
- Previous vaccination with Prevenar 13 confounds the Pneumovax 23 vaccine test in some patients. It is useful to look at the pure polysaccharide serotypes in these patients.
- The results of diagnostic vaccination studies are highly variable and should be interpreted within the appropriate clinical context. Unexpected results should be confirmed with a repeat test.
- The correct approach to testing and interpretation ensures timely diagnosis and appropriate interventions to control frequent infections and improve patient outcomes.

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