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BEST PRACTICE

Anti-pneumococcal antibody titre measurement: what useful information does it yield?

Paul Balmer, Andrew J Cant, Ray Borrow

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Measuring and interpretation of the immune response to pneumococcal polysaccharides is a complex field, owing to the diversity of the pneumococcal polysaccharide capsular types, different vaccine formulations including both polysaccharide and conjugate vaccines, diverse pneumococcal serological assays, lack of immunogenicity data for the conjugate in a number of at-risk groups and complex vaccine schedules. Even the reasons for performing pneumococcal serology can be complex, as assays may be performed for one of two reasons: either to assess an individual's immune status to the pneumococcus or to discriminate between normal and abnormal humoral immunity. This review details a history of the pneumococcal serological assays and provides some insight into when serology can prove useful, including vaccination data for certain at-risk groups.

See end of article for authors' affiliations

Correspondence to: Dr R Borrow, Vaccine Evaluation Unit, Health Protection Agency North West, Manchester Laboratory, Clinical Sciences Building, Manchester Royal Infirmary, Manchester M13 9WZ, UK; ray.borrow@hpa.org.uk

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Infections caused by pneumococci are a major cause of morbidity and mortality worldwide. Pneumonia, febrile bacteraemia and meningitis are the most common manifestations of invasive pneumococcal disease (IPD), whereas bacterial spread within the respiratory tract may result in middle-ear infection, sinusitis or recurrent bronchitis. Compared with invasive disease, the non-invasive manifestations are usually less severe, but considerably more common. Although all age groups may be affected, the highest rate of pneumococcal disease occurs in young children when immune responsiveness to sugar-protein moieties on the surface of pneumococci is poorly developed, and in the elderly population when it has waned. In addition, individuals suffering from a wide range of chronic conditions and immune deficiencies are at increased risk, in particular partial or complete antibody deficiency, complement defects, and congenital or acquired asplenia. The capsular polysaccharides of *Streptococcus pneumoniae* represent a diverse group of polymers that play an essential role in the virulence of the bacterium, with 90 serologically distinct capsules now recognised.[1]

The polyvalent polysaccharide vaccine (Pneumovax) contains 25 μg of the capsular polysaccharides of 23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F; 23vPPV) that are currently responsible for the vast majority of invasive pneumococcal infection. However, the usefulness of this vaccine is limited owing to the T-cell-independent nature of the immune response to polysaccharide antigens and its consequent failure to induce long-term protection and lack of efficacy in children <2 years of age. As a result, it is licensed for use only in individuals aged >2 years.[2]

A 7-valent pneumococcal saccharide–protein conjugate vaccine (7vPCV; Prevenar) has recently been licensed, which has been shown to be highly immunogenic in young children with an estimated efficacy of 97.3% against vaccine serotypes following three doses.[3] The 7vPCV contains approximately 2 μg of saccharide from serotypes 4, 9V, 14, 18C, 19F and 23F and 4 μg from serotype 6B. The seven serotypes included in Prevenar are among the most prevalent of those causing IPD in the targeted age group (<5 years of age), and, although universal immunisation of infants has been recommended in the US[4] for some time, the UK, since 2001, has recommended 7vPCV only for children <2 years of age in high-risk groups.[2] In July 2006, the UK Department of Health (DH) announced the introduction of the 7vPCV to the routine infant immunisation schedule, with three doses to be given at 2, 4 and 13 months from 4 September 2006.[5] A catch-up campaign for those aged up to 2 years of age will also be implemented. For persons aged ⩾65 years, a single dose of 23vPPV is recommended and patient groups at risk of IPD are recommended to be immunised according to the guidelines recommended by the DH.[6] The at-risk groups include those with anatomical or functional asplenia; chronic renal disease or nephrotic syndrome; immunodeficiency or immunosuppression due to disease or treatment (including HIV infection); chronic heart, lung and liver disease; and diabetes mellitus. Previous IPD irrespective of serotype is not considered a risk factor, and hence pneumococcal immunisation is not generally recommended. An enhanced surveillance programme has been put in

Abbreviations: CP, C-polysaccharide; HAART, highly active antiretroviral therapy; IPD, invasive pneumococcal disease; RIA, radioimmunoassay; PPV, pneumococcal polysaccharide vaccine; 7vPCV, 7-valent pneumococcal conjugate vaccine; 23vPPV, 23-valent pneumococcal polysaccharide vaccine

place, which will monitor the impact of the 7vPCV on IPD and follow up potential vaccine failures.[7] For children in birth cohorts targeted for routine or catch-up immunisation with confirmed IPD, vaccinated is dependent on their prior vaccine history and infecting serotype. If unvaccinated or partially vaccinated, a primary/booster schedule of 7vPCV should be offered. If fully vaccinated and a true vaccine failure, a further dose is offered, whereas for non-vaccine serotype cases or those where the serotype is not known, the decision to re-vaccinate is based on the pneumococcal serotype-specific serology.[8]

Both 23vPPV and 7vPCV are considered safe vaccines based on clinical experience since 1977 and 2000, respectively. Local reactions at the site of injection, such as mild soreness, swelling and redness, are observed following administration of both vaccines, along with low-grade fever. More severe systemic reactions are infrequent.[4 6 8]

SEROLOGICAL ASSAYS

Pneumococcal serological assays are performed for two main reasons. Firstly, an individual's immune status to the pneumococcus is assessed by seeing whether seroconversion occurs following vaccination, and so determining whether he/she is protected or requires vaccinated, and, if so, whether with 23vPPV or with 7vPCV (eg, for asplenia and chronic renal disease). Secondly, serology is performed to discriminate between normal and abnormal humoral immunity by using the 23vPPV as a T-cell-independent polysaccharide antigen (eg, for hypoglobulinaemia or a-γ-globulinaemia, Wiskott–Aldrich syndrome or DiGeorge anomaly). Depending on which question is being asked, different serological assays may be used.

Up to the 1980s, the radioimmunoassay (RIA) and indirect haemagglutination assay were utilised to measure antibody responses following pneumococcal vaccination. The indirect hemagglutination assay was reproducible but not as quantitative as the RIA.[9 10] Although sensitive and reproducible, the RIA has several disadvantages—namely expense, inability to differentiate immunoglobulin isotypes, and safety issues with the use of radiolabelled polysaccharides.

Over the past 30 years, a number of ELISA methodologies for the measurement of antibodies against pneumococcal polysaccharides have been developed. Broadly, these fall into two categories depending on whether the assay was for clinical management or for vaccine trial. Until the licensure of the Prevenar in 2000 in the US,[4] only polysaccharide vaccine was available and the clinical ELISA methodologies used actual 23vPPV as antigen.[11 12] For vaccine trials, serotype-specific IgG assays were developed and utilised.[13 14] For these assays, a consensus methodology,[15] as well as internationally available standards are available. These ELISAs have now been transferred to the Luminex platform to give a rapid, multianalyte particle-based flow cytometric assay.[16 17] Pneumococcal IgG ELISA can suffer from poor specificity, as the test antigen, both 23vPPV and purified specific polysaccharide, contains both serotype-specific polysaccharides and C-polysaccharide (CP). Antibodies to the CPs are not protective,[18] and so measurement of non-functional IgG antibody to CP may give misleading results, suggesting that a patient has protective antibody-mediated immunity when in fact he or she does not have it.[19] Interestingly, the RIA was less affected than the ELISA by anti-CP antibody.[20]

Before the licensure of 7vPCV, when only 23vPPV was available, it was not deemed necessary to measure individual serotype-specific IgG concentrations for clinical management. This would have been laborious to perform and, assuming that the ELISA utilised had been absorbent to remove non-functional antibodies, gave a good indication of whether a patient had responded to vaccination and whether he or she had normal or abnormal humoral immunity.[21] However, when patients have been immunised with pneumococcal conjugate vaccine, use of vaccine, whether 23vPPV or 7vPCV, as the assay antigen is not so appropriate. One small study compared an ELISA using pneumococcal polysaccharide vaccine (PPV) as antigen with a serotype-specific IgG assay, and demonstrated that only the latter could discriminate responders.[22] The lack of specificity of the 23-valent assay may be because of the suboptimal expression of the 7 serotype polysaccharides in question on the ELISA plate. The added benefit of the serotype-specific IgG ELISA was also demonstrated by Uddin *et al*.[23] Children with a history of recurrent or severe bacterial infections received two doses of 7vPCV followed by a single dose of 23vPPV; their specific IgG against two of the serotypes not covered by the 7vPCV but only by the 23vPPV remained low. The 23-valent assay was, however, of use as a simple screen for the distinction between normal and abnormal immunity.

The use of 23vPPV as an example of a T-independent polysaccharide antigen for the investigation of individuals with abnormal immunity became problematic following the DH recommendation for use of 7vPCV in at-risk groups,[24] and will become virtually inoperative following the introduction of 7vPCV into the infant immunisation schedule in 2006–7.[5] If subjects are already primed by a pneumococcal polysaccharide–protein conjugate vaccine, later administration of a polysaccharide should elicit an anamnestic-type response. Thus, the polysaccharide will not behave as a classical T-independent antigen. This could be overcome by the immunological investigation of pneumococcal serotypes that are not contained within the 7vPCV but are in the 23vPPV, such as serotypes 1 and 5. Caution should be exercised if a serotype is selected that is represented in the 7vPCV by another serotype such as 6A or 6B, because antibodies to one have been shown to cross-react with the other,[25 26] or even other bacterial capsules such as pneumococcal serotype 6B with *Haemophilus influenzae* type b.[27] An alternative approach would be to use a different polysaccharide vaccine altogether. Polysaccharide vaccines from meningococcal serogroup C and *H influenzae* type b are not suitable, as conjugate vaccines containing antigens from these pathogens are widely utilised. Other serogroups of the non-conjugated meningococcal quadrivalent polysaccharide vaccine could be utilised as, to date, Menactra, the only licensed quadrivalent meningococcal A, C, Y and W135 conjugate vaccine, is licensed only in the US.[28] Of the meningococcal serogroups, Y and W135 capsular polysaccharides may be suitable, although the appropriateness of serogroup A is in question, as this serogroup polysaccharide is not a classical T-independent antigen.[29] Alternatively, *Salmonella typhi* Vi capsular polysaccharide vaccine could be utilised and immunoassays have been developed for quantitation of antibody to this antigen.[30] However, it must be remembered that *S typhi* conjugate vaccines are under development.[31]

PROTECTIVE LEVELS AND NORMAL RANGES

Defining protective antibody levels and even normal ranges for pneumococcal IgG antibody is problematic, as the protective level may be different for different serotypes and may also vary with age. Numerous attempts have been made to assign normal ranges[11] and protective levels,[3] and define tools for the interpretation for clinical management.[19]

In infants receiving the 7-valent conjugate vaccine at 2, 4 and 6 months, ⩾97% achieved ⩾0.15 μg/ml of anticapsular antibodies for all serotypes within the vaccine.[3] This correlated with the observed protective efficacy of 97.3%. From this, a serotype non-specific threshold concentration of 0.20 μg/ml was obtained with the support of additional factors.[32] Firstly, an

antibody concentration ⩾0.20 μg/ml corresponds to the threshold of opsonic antibody titre of 1:8. Secondly, the threshold concentration also seems to predict the age-specific population disease rates, with the rates increasing when passively acquired antibody concentrations fall below 0.20 μg/ml, but decreasing when naturally acquired antibody concentrations rise above this concentration. Thirdly, the threshold is consistent with available data from passive immunisation studies on the level of bacterial polysaccharide immune globulin needed to prevent pneumococcal otitis media[33] and IPD.[34] This threshold seems to clearly discriminate between conjugate vaccines and controls in immunogenicity studies.[32] Recently, the World Health Organization has proposed a threshold of 0.35 μg/ml for use with the serotype-specific assay with CPs but not with 22F as adsorbent.[35] This has been reported to correlate with a threshold of 0.20 μg/ml when using both CPs and 22F as adsorbents, although this is still under debate for sera from infants.[36 37]

Scientifically, a threshold derived from vaccine efficacy in a population(s) cannot be used to precisely discriminate individual people at risk from those who are not. There are numerous examples of individual-breakthrough cases occurring despite protective pre-existing antibody concentrations, for a number of different pathogens,[38] although in some cases disease may be attenuated. It is likely that differences in exposure, microbial virulence and various host factors including innate immunity and concurrent viral infections make an individual's risk difficult to predict precisely.

An algorithm has been designed to attempt to generate manageable data in the form of an overall susceptibility of infection to the pneumococcal serotypes included in the serotype-specific assay. The prevalence of each of the serotypes in defined age groups was combined with the then-putative protective level of 0.15 μg/ml to generate a percentage susceptibility to infection.[19] A threshold of ⩾10% overall susceptibility on the serotype-specific assay is taken to indicate the need for intervention such as vaccination if appropriate.[19]

It is not possible to assign protective thresholds for the assays that utilise 23vPPV as antigen, as these assays have never been utilised in efficacy studies or contrasted to the functional opsonic assays. However, a number of papers do describe normal ranges or ranges that may discriminate between normal and abnormal immunity.[11 23 39] Given that little effort has been made with regard to the interlaboratory standardisation of these 23vPPV assays, there are no national or international calibrant materials available, and, as they are normally endpoint titre assays, a normal range from one laboratory could not be translated to that of another.

IMMUNOGENICITY OF 7vPCV AND 23vPCV IN AT-RISK GROUPS

Given the relatively recent licensure of 7vPCV, there are still few data on its immunogenicity in different risk groups and also on the effect of polysaccharide vaccination either before or after 7vPCV vaccination. Serological analysis following 7vPCV is therefore advisable in these groups.

In the UK, it is currently recommended that ''at-risk'' children <5 years of age are given a single dose of 23vPPV after the second birthday following on from the recommended primary course of 7vPCV for their age group.[6] Although this approach is well tolerated, few studies have been performed with the current serotype-specific assays to determine the added potential advantage of using 23vPPV to broaden coverage. One concern is that there is some evidence from studies with meningococcal vaccines that administration of polysaccharide vaccine following immunisation with conjugate vaccine may interfere with the memory B cell pool.[40] This may be of particular interest in the northern territory of Australia, where 7vPCV is administered in a primary series at 2, 4 and 6 months of age, with only indigenous children receiving a 23vPPV booster at 18 months.[41]

Studies of response to meningococcal vaccines suggest that administration of polysaccharide vaccines may reduce the magnitude and persistence of antibody responses to subsequent conjugate vaccines.[42] Both of these need to be studied for pneumococcal vaccines, now that there are immunoassays capable of discerning what interactions are occurring. The latter is of particular importance in that 23vPPV is utilised as a polysaccharide probe to ascertain whether a patient is able to respond generically to polysaccharides or not. Serological data are available which demonstrate that an initial dose of 23vPPV in subjects aged ⩾70 years leads to a decreased antibody response to subsequent 7vPCV.[43] No published data are available for children.

Although hyporesponsiveness has been clearly demonstrated after repeated doses of meningococcal group C polysaccharide vaccine,[44–46] this question is more complex following repeated doses of 23vPPV. In the 1970s, in a study of a 12-valent PPV given either as two doses in infancy at 3–5 months and at 9–11 months or as a single dose at 6–12 months of age, antibody levels of 9 of the 10 serotypes assayed for were lower at 1 month following two doses as opposed to a single dose.[47] In a study of patients undergoing treatment for community-acquired pneumonia who were vaccinated with 23vPPV and re-vaccinated 4–7 years later, vaccinated resulted in significant increases in antibody concentration, although to lower levels than after primary vaccination for all six serotypes assayed for (1, 4, 7F, 14, 18C and 19F).[48] Recent data from the US demonstrate that a second dose of 23vPPV is immunogenic in older adults and that the antibody levels remain increased relative to baseline for 5 years after vaccination.[49] Interestingly, for the four serotypes studied, serotype-specific IgG geometric mean concentrations were either similar (serotypes 3 and 23F) or lower (serotypes 4 and 14) in the vaccinated groups. Few data following repeated vaccination with 23vPPV in children are available. In a study in toddlers of multiple regimens of an investigational pneumococcal conjugate and 23vPPV, a non-randomised comparison of a single dose of 23vPPV as compared with two doses of 23vPPV, given 12 months apart, was possible.[50] Following two doses of 23vPPV, antibody levels to serotype 14 were higher than following a single dose, but lower for 23F. For the other five serotypes assayed, antibody levels against serotype 19F were potentially higher following two doses as opposed to one, potentially lower for serotypes 4, 6B and 9V and similar for serotype 18C. Therefore, hyporesponsiveness following repeated doses of 23vPPV has been demonstrated for certain serotypes but there is little agreement between studies. More importantly, the clinical relevance of this is not known.

For the clinician, 7vPCV is an obvious choice in infection-prone subjects who are non-responders to the 23vPPV. Studies have demonstrated that the 7vPCV is more immunogenic than the 23vPPV in this group, although children did show a significantly lower post-vaccination antibody levels than healthy controls previously naive to pneumococcal vaccination.[51] It is an interesting question whether these children would have responded in a manner more similar to the controls if they had not received 23vPPV earlier. A different study in adults again demonstrated the immunogenicity of 7vPCV in adults with specific antibody deficiency; however, two doses were required to mount a suitable response.[52]

Invasive pneumococcal infection is still a prevalent disease among the HIV-infected population in certain countries, even in the era of highly active antiretroviral therapy (HAART).[53] Several studies have assessed the antibody response of

Take-home messages

- Measurement of pneumococcal-specific antibodies provides the assessment of immunity to the pneumococcus or investigation of a potential immunodeficiency.
- Interpretation of the data should always be related to the clinical history that prompted the request for testing.
- The introduction of the seven-valent pneumococcal conjugate vaccine into the infant immunisation schedule and the increased use of this vaccine in at-risk patient groups will result in more pneumococcal serology being performed.
- For assessment of response to the conjugate vaccine, the assay used needs to be serotype-specific.
- Measurement of anti-pneumococcal antibodies will also increase the knowledge of the immunogenicity of the conjugate vaccine in at-risk patient groups, for which at the moment there is limited data available.

HIV-infected individuals to 23vPPV; in the pre-HAART era, most studies showed an impaired response compared with healthy controls.[54–57] By comparison, HIV infected individuals receiving HAART responded to 23vPPV at least as well as healthy individuals.[58] Immunisation of HIV-1-positive infants ($n = 30$) with three doses of 7vPCV was found to be immunogenic, with 88–100% achieving a fourfold rise in serotype-specific IgG levels depending on serotype; 95% had a level ≥ 0.15 μg/ml.[59] At 15 months of age, antibody levels to the seven serotypes were still above baseline levels and approximately 70% had a fourfold rise following administration of a booster, except for serotype 14, to which only 39% had a fourfold rise, but this was probably owing to the higher pre-booster levels than the other serotypes. A study in 14 Greek symptomatic HIV-1 children (median age 128 months), however, demonstrated lower serotype-specific geometric mean antibody concentrations following two doses of 7vPCV as compared with the 21 age-matched controls, and predicted that long-term effectiveness was expected to be reduced among infected children.[60]

Few data are currently available on antibody responses following 7vPCV dose in patients with haematological diseases. Patients undergoing haematopoietic cell transplantation are at increased risk for infections with *S pneumoniae*[61] and have long-lasting, impaired antibody responses to PPVs.[62] Molrine *et al*[63] randomised non-T-cell-depleted sibling allogenic recipients for pre-transplantation immunisation with 7vPCV. This was for both the donor and for the recipient with vaccination 7–10 days pretransplant. All patients then received 7vPCV at 3, 6 and 12 months. Significantly higher antibody levels were detected at all time points up to 12 months for the group which had received pretransplantation vaccination. After 12 months, with the exception of serotype 6B, the antibody levels were similar. A further study[64] explored the use of pretransplant immunisation with 7vPCV in patients undergoing autologous transplantation. Vaccination with 7vPCV was given at a mean of 10 days before autologous stem cell collection in those randomised to receive precollection vaccination. Vaccination was again at 3, 6 and 12 months and, up to 12 months, the antibody levels were significantly higher in those who had received 7vPCV before transplantation. On the basis of these two studies, three doses of 7vPCV starting 6–12 months after transplantation are now recommended by the European Group for Blood and Marrow Transplantation for small children and patients undergoing stem cell transplantation.[65]

The loss of splenic function is associated with an increased risk of life-threatening pneumococcal infection. Immunological responses to 23vPPV in asplenics vary; healthy patients who have undergone splenectomy owing to trauma gave responses similar to those of controls, whereas patients with an underlying immunological disease as well as healthy asplenic patients did not respond.[66] Few data exist in asplenics following 7vPCV vaccination. One small study in children demonstrated that protective antibody levels induced by 7vPCV varied according to serotype, ranging from 100% response for serotypes 18C and 19F to 40% for serotype 6B.[67] Another report detailed two patients who had recurring pneumococcal bacteraemia after undergoing splenectomy despite having received numerous doses of 23vPPV. Following the administration of 7vPCV, high levels of serotype-specific IgG were induced to the seven serotypes.[68] A study in 39 near or totally splenectomised patients with hereditary spherocytosis, aged 5–27 years, demonstrated that receiving a single dose of 7vPCV showed significant rises in the serotypes contained within 7vPCV.[69]

Children with nephrotic syndrome, renal allografts or chronic renal insufficiency have an increased risk of pneumococcal disease owing to urinary loss of immunoglobulin, immune suppression from their disease and/or drug therapy. A review of 26 published studies of 23vPPV in this population demonstrated serological responses by the majority of patients to at least some pneumococcal serotypes, and the use of steroids did not alter this response. In the studies with >6-month follow-up, declining antibody titres were consistently reported, and this decline was usually more rapid than in healthy controls.[70] Very few data are available on responses to 7vPCV in this patient group. A small study has shown that, in children >5 years of age, 7VPCV was immunogenic and that immune suppression did not influence the antibody response.[71] However, there is a report from the US of vaccine failure in a 7-year-old due to serotype 23F 2 months after a single dose of 7vPCV.[72] The population-based surveillance in which this was highlighted also cited vaccine failure with comorbid conditions including chronic lung disease, leukaemia, heart disease and HIV, and also described non-traditional comorbidity features that were associated with cases of IPD such as asthma, eczema, seizure disorder, central nervous system disease, prematurity, lupus and metabolic disorder.[72]

THE FUTURE

With the recommendation of the use of Prevenar in at-risk groups aged <5 years in 2004[24] and its imminent introduction into the UK infant immunisation schedule,[7] it is anticipated that the need for pneumococcal antibody testing will increase, in particular during the enhanced surveillance period following the introduction of Prevenar. Also, until more experience is gained with the use of 7vPCV in various at-risk cohorts, serological monitoring of patients is advised to obtain further knowledge of the immunogenicity of the pneumococcal conjugates.

Authors' affiliations

Paul Balmer, Ray Borrow, Vaccine Evaluation Unit, Health Protection Agency North West, Manchester Laboratory, Manchester Royal Infirmary, Manchester, UK

Andrew J Cant, Department of Paediatric Immunology, Newcastle General Hospital, Newcastle upon Tyne, UK

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