October/November 2019 Edition no. 62

AMPATHCHAT

Dr Cathy van Rooyen, Pathologist, Immunology
Dr Sylvia van den Berg, Clinical Pathologist, Immunology

Pneumococcal serotype-specific antibody testing

Patients suffering from recurrent infections, autoimmunity or lymphoproliferative disease may have an underlying humoral immunodeficiency. Immunodeficiencies may develop due to primary (i.e. hereditary) or secondary causes, e.g. malignancies, immunosuppressant therapy, acute and chronic infections, including HIV, protein-losing conditions, etc.

Patients with impairment in their B cell function or maturation, with or without T-cell dysfunction, will have aberrant antibody production. This may manifest as:

- Severe hypogammaglobulinaemia (i.e. X-linked agammaglobulinaemia);
- Reduction in total immunoglobulins with decreased IgG, IgA and or/IgM (i.e. common variable immunodeficiency or CVID);
- Reduction in total IgA (IgA deficiency);
- Reduction in IgG subclasses (IgG subclass deficiency); or
- Normal immunoglobulin levels with diminished antibody responses to polysaccharide antigens following vaccination (i.e. specific antibody deficiency (SAD), CVID, IgA deficiency or IgG subclass deficiency).

Patients with a pure T-cell defect will also have abnormal antibody production due to a lack of T-cell help for antibody production.

Clinical manifestations of impaired B-cell function include recurrent upper and lower respiratory tract infections, specifically with encapsulated bacteria, including *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. They may also suffer from prolonged *Giardia lamblia* gastrointestinal tract infections, enteroviral infections, *Staphylococcus aureus* infections and unusual infections with *Mycoplasma* and *Ureaplasma* organisms, among others. Occasionally patients may present primarily with autoimmune manifestations, and not predominantly infections. The most common of these are autoimmune cytopaenias. Malignancies, granulomatous disease and lymphoid hyperplasia may also be the presenting problem in patients with CVID.

The warning signs regarding infections that may alert to possible PIDs can be summarised by the acronym SPUR. Patients presenting with infections that are:
Severe
Persistent
Unusual
Recurrent
should be investigated for a possible PID.
Humoral immunodeficiencies that affect the quantity and quality of antibody production are the most common immunodeficiencies. The IgA deficiency has an incidence of 1:300, and CVID has an incidence of 1:10 000. Humoral immunodeficiencies affect children and adults, and are often only diagnosed in adulthood and even at advanced age. Laboratory investigations should include IgG (with or without subclasses), IgA, IgM and evaluation of specific antibody production. Secondary causes for decreased immunoglobulin levels should always be excluded first.

Assessment of vaccine responses to evaluate specific antibody production

Vaccine responses should be determined in all patients with a suspected humoral immunodeficiency with IgG levels >1g/ℓ, regardless of whether the suspected cause is primary or secondary. This evaluation should always be performed before initiation of any immunoglobulin replacement therapy, as results are not reliable after the initiation of immunoglobulin replacement therapy. Antigen-specific IgG levels, or the so-called vaccine responses, can be variable in patients with a dysfunctional humoral immune system and should be followed longitudinally. A normal level on one occasion does not exclude an immunodeficiency and should be evaluated at a later stage for waning antibody levels.

Vaccine responses should be determined in all patients with a total IgG >1g/ℓ, except in cases in which the treating physician judges that delay in treatment will be detrimental to the patient. In practice, vaccine responses are evaluated by means of measuring IgG levels to known vaccine antigens or documented infection (i.e. measles), and should include T-cell-dependent responses to a protein antigen (i.e. Tetanus toxoid) and a T-cell-independent response to polysaccharide antigens (*S. pneumoniae*). Measurement of antibodies to routine childhood vaccines, including measles, mumps, rubella, varicella and Hepatitis B, may be helpful.
Antibodies must be measured pre-immunisation and four weeks post-immunisation and are measured to polysaccharides (*S. pneumoniae*) and protein antigens (Tetanus toxoid). A patient may respond poorly to one or both types.
If the antibody level is below the protective limit for tetanus toxoid (<0.10 IU/mℓ) after vaccination, an impaired response can be assumed. Some patients with CVID can make protective antibodies to Tetanus toxoid , but still need immunoglobulin replacement therapy to protect against other bacterial infections.
Specific antibody responses to pure pneumococcal polysaccharide antigens have previously been determined by measuring total *S. pneumoniae* IgG levels . This level did not take into account variable responses to individual *S. pneumoniae* serotypes and lacked specificity and sensitivity in evaluating true polysaccharide responsiveness. International criteria have been developed for the diagnosis of functional humoral immunodeficiencies, which require the measurement of antibodies to various *S. pneumoniae* serotypes. The Luminex assay, a multiplex bead immunoassay, has been

evaluated and validated at Ampath laboratories to determine antibodies to different *S. pneumoniae* serotypes simultaneously. This technology has enabled Ampath to implement the measurement of antibodies against 13 different *S. pneumoniae* serotypes. The *S. pneumoniae* serotypes in the assay are the 13 serotypes present in the pneumococcal conjugate vaccine, Prevenar®.

Interpretation of test results are based on four response phenotypes. These have been summarised in Table 1.

Table 1: Summary of PPV23-deficient response phenotypes

Phenotype*	PPV23 response, age >6 years	PPV23 response, age <6 years	Notes
Severe	≤2 protective titres (≥1.3 µg/mℓ)	≤2 protective titres (≥1.3 µg/mℓ)	Protective titres present are low
Moderate	<70% of serotypes are protective (≥1.3 µg/mℓ)	<50% of serotypes are protective (≥1.3 µg/mℓ)	Protective titres present to ≥3 serotypes
Mild	Failure to generate protective titres to multiple serotypes or failure of a twofold increase in 70% of serotypes	Failure to generate protective titres to multiple serotypes or failure of a twofold increase in 50% of serotypes	Twofold increase assumes a pre-vaccination titre of less than cut off values of 4.4–10.3 µg/mℓ, depending on serotype
Memory	Loss of response within six months	Loss of response within six months	Adequate initial response to ≥50% of serotypes in children under six years of age and ≥70% in those over six years of age.
* All phenotypes assume a history of infection			
PPV23: Pneumococcal polysaccharide vaccine (Pneumovax 23®) Reference: Orange, JS, Ballow, M et al. 2012. 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 and Immunology. *Journal of Allergy and Clinical Immunology* 130:S1–24.			

A pneumococcal serotype-specific antibody level of 1.3 ug/mℓ or higher is considered to be protective following polysaccharide immunisation. If the baseline level is >1.3 ug/mℓ, a twofold response is considered adequate. In levels below the protective level, a response above the protective level is required. In high baseline levels above the protective level, a twofold elevation may not always be obtained. In addition to individual serotype-specific criteria, a protective response in more than 50% of the serotypes in children under six years and a more than 70% serotype response in patients over six years is required. Patients with a mild response phenotype do not respond to sufficient serotypes. Patients with a moderate response respond to at least three serotypes. Patients with a severe phenotype produce protective antibodies to two or fewer serotypes. In the memory phenotype, patients respond adequately initially, but after six months, protection is not sustained in >50% of serotypes in patients under six years and in more than 70% of serotypes in patients over six years. Therefore, if a normal response is obtained, follow-up antibody levels are recommended in six months' time to assess whether there is waning immunity.

Non-responsiveness, poor responsiveness or accelerated waning immunity could be in keeping with a dysfunctional immune system. These should be interpreted in conjunction with the immunoglobulin levels, memory B-cells and clinical picture.

The following vaccines are recommended:
- Age under 2 years: DTPa-hepB-IPV-Hib vaccine (i.e.Hexaxim®) and Prevenar®
- Age 2–6 years: DTacP-ipv combined vaccine (i.e. Tetraxim®) and Pneumovax 23®, not Prevenar®
- Age over 6 years: dTacP-ipv combined vaccine (i.e Adacel Quadra®) and Pneumovax 23®, not Prevenar®

At Ampath laboratories, specific antibody responses will be assessed by measuring Tetanus toxoid IgG (to assess T-cell dependent responses) and 13 *S. pneumoniae* serotype-specific antibodies (to assess T-cell independent responses). A baseline pre-vaccination measurement, four-week post- vaccination measurement and six-month post-vaccination measurement is advised.

As pneumococcal serotype-specific antibodies provide better evaluation of the patient's humoral immune function, the Diptheria toxoid IgG and *H. influenzae* IgG assays will be discontinued in lieu of the superior multiplex pneumococcal antibody assay. The cost of the new pneumococcal serotype-specific IgG antibody panel (13 serotypes) and Tetanus toxoid IgG antibodies will not be significantly more expensive than the four specific antibodies (Tetanus toxoid IgG, Diptheria toxoid IgG, *H. Influenzae* IgG and total *S. pneumoniae* IgG) that formed part of the previous specific antibody panel. However, much more valuable information will be obtained, which will enable more accurate diagnosis, as well as the exclusion of functional humoral immunodeficiencies. The accurate diagnosis of humoral immune deficiencies is essential to guide therapy, which may include immunoglobulin replacement therapy or prophylactic antibiotics.

SUMMARY

Indication
Diagnosis of functional humoral immunodeficiencies, which may be primary or secondary.

Interpretation
Pre-vaccination serotype-specific antibodies are compared to four-week and six-month post-vaccination levels. Vaccination with pneumococcal polysaccharide vaccine (Pneumovax 23®) is required to assess post-vaccination pneumococcal antibodies in patients over two years. If an adequate response is not obtained or unacceptable waning is noted, a functional humoral immunodeficiency can be diagnosed according to diagnostic criteria for primary immunodeficiencies found at https://esid.org/.

Other accompanying tests
The test should be requested with Tetanus toxoid IgG antibodies, total IgA, IgM and IgG levels with or without IgG subclasses. Memory Bcells can support the diagnosis of CVID or IgG subclass deficiency and help to predict morbidity. Lymphocyte subsets may also be indicated to assess B-cell numbers (important in the diagnosis of X-linked agammaglobulinaemia) and T-cell numbers and subsets (to exclude a concomitant T-cell defect).

Specimen collection
One single clotted tube (SST tube) is required.

Test mnemonics
- PNEUMOPRE for baseline *S. pneumoniae* serotype-specific antibodies
- PNEUMOPOST for four-week and six-month post-vaccination levels

References available on request