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Quantitative serum immunoglobulin tests

This article forms part of our 'Tests and results' series for 2013, which aims to provide information about common tests that general practitioners order regularly. It considers areas such as indications, what to tell the patient, what the test can and cannot tell you, and interpretation of results.

Keywords

immunologic tests

What is the test?

Immunoglobulins are protein molecules. They contain antibody activity and are produced by the terminal cells of B-cell differentiation known as 'plasma cells'. There are five classes of immunoglobulin (Ig): IgG, IgM, IgA, IgD and IgE. In normal serum, about 80% is IgG, 15% is IgA, 5% is IgM, 0.2% is IgD and a trace is IgE.¹

Quantitative serum immunoglobulin tests are used to detect abnormal levels of the three major classes (IgG, IgA and IgM). Testing is used to help diagnose various conditions and diseases that affect the levels of one or more of these immunoglobulin classes. Some conditions cause excess levels, some cause deficiencies, and others cause a combination of increased and decreased levels. IgD and IgE will not be discussed in this article.

When should the test be ordered?

Serum immunoglobulin tests are used for the evaluation of antibody (humoral) immunodeficiencies. A low level of immunoglobulin is termed 'hypogammaglobulinaemia'. The tests should be ordered if a patient has symptoms suggestive of an immunoglobulin deficiency, such as family history of immunodeficiency, recurrent or severe or unusual bacterial infections, lack of response to antibiotics, unusual or recurrent viral infections and/or chronic unexplained diarrhoea. Patients with antibody deficiency are particularly predisposed to recurrent sinopulmonary

infections, especially with polysaccharide encapsulated organisms including *Streptococcus pneumoniae* and *Haemophilus influenzae*. There are many types of antibody deficiencies ranging from isolated IgA deficiency to severe deficiencies of all immunoglobulins.² These may occur as an isolated defect or as part of a wider combined immunodeficiency affecting both T- and B-cells.

Serum immunoglobulin tests may be used in the assessment of conditions associated with chronic inflammation (eg. rheumatoid arthritis, systemic lupus erythematosus, autoimmune liver disease) or chronic infections (eg. hepatitis C, HIV).³ The tests should also be ordered along with other tests, including serum and urine electrophoresis, when there is suspicion for B-cell malignancy such as myeloma, lymphoma or chronic lymphoid leukaemia (CLL).

Measurement of IgM in a newborn may assist in the diagnosis of a congenital or neonatal infection.

Depending on the condition (eg. myeloma), serum immunoglobulin tests may also be ordered periodically to monitor disease progression.

When shouldn't the test be ordered?

Otherwise healthy thriving children with frequent 'colds' do not need their immunoglobulin levels measured. In Australia, normal healthy toddlers can have up to 12 infections a year. The number of infections increases if the child attends childcare, has older siblings and/or parents who smoke.

How does the test work?

Nephelometry and turbidimetry are the most widely used methods because of their speed, ease of use and precision. In both nephelometry and turbidimetry, a light source is projected through a liquid sample within a transparent container. Turbidimetry measures the decrease in the intensity of light and nephelometry measures scatter of light as it passes through the sample,

which is proportional to the concentration of the immunoglobulin in the solution. Laboratory levels can vary by laboratory due to differences in testing technique.

What do the results mean?

IgG, IgA, and IgM levels are usually evaluated together. Abnormal test results indicate that there is something affecting the immune system and may suggest the need for further testing. Abnormal Igs are not diagnostic but can, in association with an appropriate clinical history and other tests results, be a strong indicator of a disease or condition.

There are a number of conditions associated with decreased and increased immunoglobulin levels.

Immunoglobulin deficiency

- Primary (inherited) – less common disorders in which the body is not able to produce one or more class of immunoglobulin
- Secondary (acquired) – the most common causes of hypogammaglobulinaemia result from an underlying condition that either affects the body's ability to produce Igs or increases the loss of Igs from the body (Table 1).

Hypogammaglobulinaemia is more frequently due to secondary rather than primary causes.⁴ Secondary causes include nephrotic syndrome, protein losing enteropathies, sepsis and malignancies (Table 1). For patients who have loss of immunoglobulin into the renal tract or gut, the

IgM tends to remain normal as it is retained due to its large size.

There are a large number of primary humoral immunodeficiencies in which immunoglobulins show a mild or profound alteration (usually decreased, sometimes increased).⁵ Selective IgA deficiency is one of the most common immunodeficiencies and occurs with a frequency of 1:500.⁵ Laboratory testing shows undetectable IgA, and normal IgG and IgM. This finding is often found incidentally, such as when testing for coeliac disease. Most patients are asymptomatic, while some can have recurrent sinopulmonary infections in later childhood. As serum levels of IgA do not reach adult levels until the age of 8 years, a diagnosis of IgA deficiency should not be made in children less than 4 years of age.

IgG levels fall in the first 6 months of life as maternal transplacentally acquired antibody level falls (normal physiological hypogammaglobulinaemia). Premature infants have less maternal IgG and may reach a lower nadir. In some cases, the normal gradual increase in the infant's IgG in the first year of life is delayed (so-called transient hypogammaglobulinaemia of infancy).⁵ The Ig levels usually recover by the age of 2 years, although this can be delayed until the child is up to 8 years of age. In these patients, the IgM is usually normal.

Patients with X-linked agammaglobulinaemia usually have low or undetectable levels of all the major immunoglobulins due to abnormalities of B-cell development, leading to absent or markedly

reduced B-cell numbers.⁵ Symptoms usually first appear between 4–8 months of age as levels of IgG acquired transplacentally from the mother decline.

Common variable immunodeficiency is the most common severe antibody deficiency affecting both children and adults.⁵ It has a variable age of onset, usually occurring by the third decade, although there is often a significant delay between symptoms and diagnosis. These patients have hypogammaglobulinaemia (low IgG with low IgA and/or IgM), poor specific antibody responses to vaccinations, and an increased incidence of autoimmune disease, particularly autoimmune cytopenias due to dysregulation of the immune response.

Immunoglobulin excess

Causes of increased immunoglobulin levels are:

- increased polyclonal immunoglobulins resulting from many different immune (plasma) cells
- increased monoclonal immunoglobulins resulting from proliferation of a single clone of plasma cells.

These causes are further explained in Table 2.

An increase in immunoglobulins (hypergammaglobulinaemia) is usually polyclonal and related to immune activation associated with autoimmune diseases or infection. In these cases, multiple Ig classes tend to be affected. In contrast, monoclonal production is usually associated with a marked increase in one class of immunoglobulin only (referred to as a paraprotein or monoclonal protein). This may be associated with a decrease in the other two classes (immunoparesis). Although patients have an increase in total immunoglobulins, they are often relatively immunocompromised because most of the immunoglobulin production is abnormal and does not contribute to the immune response.

Next steps?

The presence of hypogammaglobulinaemia should be confirmed on repeat testing. Any underlying secondary causes of hypogammaglobulinaemia should be considered before referral to a consultant immunologist for investigation of hypogammaglobulinaemia. It is important to analyse urine for protein loss. Medication history may reveal use of medications associated with low immunoglobulin levels (Table 1).

Table 1. Causes of secondary or acquired hypogammaglobulinaemia

Conditions that cause an abnormal loss or increased catabolism of immunoglobulin	<ul style="list-style-type: none"> • Nephrotic syndrome and other severe renal diseases • Severe burns • Sepsis • Protein-losing enteropathy • Intestinal lymphangiectasia
Conditions/factors affecting immunoglobulin production	<ul style="list-style-type: none"> • Nutritional due to malnutrition or alcoholism • Drugs such as phenytoin, carbamazepine, immunosuppressive drugs or chemotherapy agents • Malignancies, especially haematological malignancies (chronic lymphocytic leukaemia, lymphoma, multiple myeloma) • Rheumatological disease, including rheumatoid arthritis or systemic lupus erythematosus (SLE) • Viruses including HIV, Epstein-Barr virus, rubella, cytomegalovirus

In adults with abnormal or excessive immunoglobulin production, a serum protein electrophoresis should be performed to detect the presence of any monoclonal band (also called 'paraprotein' or 'M spike') and urine protein electrophoresis for Bence-Jones protein.

Full blood count (FBC) is important to identify lymphopenia, anaemia, and thrombocytopenia (all can occur with hypogammaglobulinaemia) and to exclude neutropenia. Lymphocytosis may be seen in CLL or lymphoma. Lymphopenia in infants with infections, diarrhoea and/or failure to thrive is an important finding as it suggests severe combined immunodeficiency. Severe combined immunodeficiency is a medical emergency, and patients should be urgently referred to specialist centres for continuation of diagnosis and treatment as soon as it is suspected (ie. if patients have low absolute lymphocyte counts and low/absent immunoglobulins).⁶

There is controversy around measuring IgG subclass levels, as isolated IgG subclass deficiency is rarely significant and, in the absence of specific antibody deficiency, has little clinical relevance. IgG subclasses should not be routinely ordered.⁷

Tests that should be ordered only after discussion with a clinical immunologist include:⁸

- identification of T- and B-lymphocytes with the use of flow cytometry

- measurement of salivary IgA. This test is useful in infants as this mucosal immunoglobulin matures before circulatory IgA. If a low serum IgA is detected in this age group, salivary IgA should be measured before diagnosing IgA deficiency
- specific antibody responses to vaccines such as tetanus and pneumococcus provide a qualitative assessment of immunoglobulin function and can assist in the diagnosis of conditions such as specific antibody deficiency. Such patients have normal immunoglobulin levels but poor specific responses to vaccines.⁹

Pitfalls

Values for all immunoglobulin levels for paediatric patients must be compared with normal laboratory values for age. The normal term infant has undetectable levels of IgA and IgM at birth and these levels rise progressively in the first 2–3 years of life. IgA levels increase with age and are often elevated in the elderly.

Patients with immunoglobulin deficiencies may have false negative results from laboratory tests that measure antibodies in the blood. For example, one test for coeliac disease detects the IgA type of anti-tissue transglutaminase antibody (anti-tTG). If a person has a deficiency in IgA, then results of this test may be negative when the person, in fact, has coeliac disease. If this is

suspected to be the case, then a quantitative test for IgA may be performed.

The impaired antibody responses to pathogens in hypogammaglobulinaemic states may make the serological diagnosis of certain infections, such as HIV and Epstein-Barr virus, difficult. In these patients, nucleic acid detection methods or culture should be performed.

Importantly, the estimation of quantitative immunoglobulins does not allow an assessment of clonality. Also, a normal immunoglobulin level does not exclude a small paraprotein and further testing should be performed if this is suspected. Paraproteins can also interfere with immunoassays and should be suspected if there are unusual or unexpected results for the clinical picture, particularly in patients with known lymphoproliferative disease.¹⁰ If the referring doctor is aware that the patient has an underlying paraprotein, this should be stated on the request form.

Who should be referred to a clinical immunologist?

The presence of hypogammaglobulinaemia should be confirmed on repeat testing. Secondary causes of hypogammaglobulinaemia should be considered and where possible excluded before referral to a consultant immunologist. Referral for hypergammaglobulinaemia will depend on the underlying cause. Patients with a monoclonal paraprotein should be referred to a haematologist for further evaluation.

Case study

Peter, 2 years of age, attended his general practitioner with a history of two lower respiratory tract infections, three ear infections and one episode of gastroenteritis over a recent winter. He is well nourished and has appropriate developmental milestones. He started attending childcare 6 months ago and has two older siblings. The respiratory tract infections all responded to 5 day courses of oral antibiotics.

The GP organised FBC and immunoglobulin levels. The FBC was normal, but immunoglobulin levels were low for IgG.

Table 2. Causes of increased immunoglobulin levels

Immunoglobulin result	Associated conditions
Polyclonal increase in any or all of the three classes (IgG, IgA and/or IgM)	<ul style="list-style-type: none"> • Infections, acute and chronic (including HIV, Epstein-Barr virus, cytomegalovirus) • Connective tissue diseases (rheumatoid arthritis, systemic lupus erythematosus, scleroderma) • Chronic active autoimmune hepatitis (IgG) • Primary biliary cirrhosis (IgM) • Haematologic disorders • Non-haematologic malignancies • In cord blood of newborns with intrauterine infection (IgM to offending pathogen)
Monoclonal increase in one class with or without decrease in other two classes	<ul style="list-style-type: none"> • Multiple myeloma (IgG, IgA, rarely IgM) • Monoclonal gammopathy of uncertain significance • Chronic lymphocytic leukaemia • Non-Hodgkin lymphoma • Waldenstrom macroglobulinaemia (IgM) • Primary systemic amyloidosis • Monoclonal cryoglobulinaemias

Does this patient have an immunodeficiency?

It is unlikely. The number of infections is still within the expected range for a healthy child attending a childcare facility and with older siblings who are additional sources of infectious agents.¹¹ None of the infections were refractory to antibiotics and no unusual or rare complications were reported. The child is not failing to thrive. FBC was normal (no evidence of lymphopenia, which can occur in more severe forms of immunodeficiency).

Should the patient have the IgG repeated immediately?

No. The patient could have transient hypogammaglobulinaemia and the IgG would be expected to increase over the next 12 months. IgG can be repeated in 6 months if the patient is still having problems with infections. If IgG is still low or dropping and there are clinical concerns, referral to a clinical immunologist for further assessment should be considered.

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