Royal United Hospitals Bath
NHS Foundation Trust

Information for Clinicians

RUH Immunology Department

Guidance on the Interpretation of Immunoglobulin Results

Introduction

Immunoglobulins (also known as antibodies) are proteins consisting of 2 heavy and 2 light chains, produced by terminally differentiated B cells, known as plasma cells. They form part of the adaptive immune system and are important in immune protection, particularly against viral and bacterial infection.

- There are 5 classes of immunoglobulin, IgG, A, M, D and E, determined by their heavy chains. In normal serum, approximately 80% is IgG, 15% IgA, 5% IgM, with 0.2% IgD and trace IgE.
- Laboratory testing for immunoglobulins in peripheral blood typically detects the 3 major classes, IgG, A and M.

Immunoglobulin levels change with age as the adaptive immune response matures. Age related reference ranges are issued on the immunoglobulin report to aid interpretation.

- IgG levels fall in the first 6 months of life as transplacentally acquired maternal antibody declines. This is a normal physiological process.
- In premature infants, nadir levels can be low as transplacentally acquired IgG is more limited.
- Transient hypogammaglobulinaemia of infancy occurs when the normal gradual increase in immunoglobulins in the first year of life is delayed. Levels usually recover by the age of 2 years, but in some cases this can take longer.
- IgA levels increase with age and are often elevated in the elderly.
- IgM levels can decline with age. An isolated low IgM, in the absence of a paraprotein, can be seen in adults over 60 and is of doubtful clinical significance.

A small percentage of otherwise healthy individuals will statistically have immunoglobulin levels that fall either just above or below the laboratory reference ranges (“normal range”).

Immunoglobulin assessment is helpful in the diagnosis of diseases that affect levels of one or more antibody class.

- High levels can be seen in certain conditions
- Deficiency (hypogammaglobulinaemia) can be seen in other conditions
- A combination of high levels of certain classes, with deficiency of other classes can occur, for example in the context of a monoclonal protein, see below.

Increased immunoglobulin levels may be

- Monoclonal, reflecting output from a clonal B cell population
- Polyclonal (reactive), reflecting output from multiple B cell populations
- Less commonly oligoclonal, reflecting increased output from a small number of B cell clones

Serum electrophoresis is required to determine whether an elevated immunoglobulin class is monoclonal or polyclonal. If a monoclonal immunoglobulin is identified, immunofixation is required to type the monoclonal (M) protein, also known as the paraprotein.

Urine or serum should be assessed for the presence of light chains in the context of a paraprotein. Most immunoglobulin light chains are bound as intact immunoglobulin molecules. The 2 isotypes of light chains – kappa and lambda, are normally secreted by bone marrow plasma cells in a 2:1 ratio. Lambda light chains are more slowly excreted by the kidney, so the serum light chain ratio is normally close to 1:1.

- Renal impairment impairs excretion of both isotypes so the serum free light chain ratio becomes closer to the 2:1 ratio.
- In an acute phase response, both kappa and lambda may be elevated, giving a normal ratio.
- A light chain ratio falling significantly outside of the normal range implies a clonal B cell disorder.
- Abnormal ratios close to the normal range are more difficult to interpret and may be benign.
- The clinical features that suggest that an abnormal free light chain may be significant are the same as those for a paraprotein, see below.

If no monoclonal protein is identified on serum electrophoresis, urine / serum light chain assessment, then elevated immunoglobulins usually reflect polyclonal increase.

Oligoclonal immunoglobulins in serum can be seen in disorders associated with immune stimulation such as chronic infection, autoimmunity and certain B cell neoplasms

Results should be interpreted in the context of the clinical history, including medication history, examination findings and other laboratory data, rather than in isolation, and whether the patient was clinically ill at the time of testing. Abnormal immunoglobulin results indicate that there is something affecting the immune system and further testing may be required.

When to test?

1. **When there is concern regarding antibody deficiency**, for example patients presenting with
   - Recurrent, severe or unusual bacterial infection
     - Patients with hypogammaglobulinaemia are predisposed to recurrent sinopulmonary infection, especially with polysaccharide encapsulated organisms such as Strep pneumonia and Haemophilus influenzae
   - Failure to respond as expected to antimicrobial therapy
   - Recurrent or unusual viral infection
   - Unexplained diarrhoea
   - Family history of immune deficiency
   - Infection in the context of iatrogenic immune suppression e.g. with steroid or Rituximab

   Hypogammaglobulinaemia can occur in isolation, but can also be associated with T cell deficiency, in which case the immune deficiency is considered “combined”. Patients with combined immune deficiency are at risk of bacterial and viral infection, but can also have opportunist infection, i.e. infection with microorganisms that do not cause infection in immune competent individuals. All such patients should be under the care of an immunologist.

   A full blood count should be undertaken to assess for lymphopenia, anaemia and thrombocytopenia. Lymphopenia in infants with infection, diarrhoea or failure to thrive suggests severe combined immune deficiency, which is a medical emergency, requiring urgent paediatric referral.

2. **When there is concern regarding a monoclonal B cell lymphoproliferative disease** e.g. myeloma, lymphoma or chronic lymphocytic leukaemia.

   - Immunoglobulins, serum and urine electrophoresis (or serum free light chains) should be tested in this context to assess for the presence of an M protein (paraprotein) and associated immune paresis (suppression of normal immunoglobulins)

- Serial monitoring in the context of a paraprotein can be used to assess disease progression, as advised for example when MGUS is identified

3. **In the assessment of conditions associated with raised globulins**, e.g. connective tissue disease, chronic liver disease and chronic infection such as untreated HIV, where hypergammaglobulinaemia is a cause of raised plasma viscosity.

Hypogammaglobulinaemia

Immunoglobulin deficiency may be

- **Primary** (inherited) and can affect one or more immunoglobulin class. Apart from selective IgA deficiency, these conditions are rare
- **Secondary** (acquired), resulting from an underlying condition or use of treatment that affects antibody production or results in increased immunoglobulin losses. Secondary antibody deficiency is much more common than primary, and should always be considered

The finding of hypogammaglobulinaemia should be repeated to confirm, particularly if the patient was unwell at the time of initial testing. Urine should be assessed for protein loss especially if there is isolated low IgG

Primary Antibody Deficiency

- **Selective IgA deficiency** is the most common primary antibody deficiency and is usually asymptomatic – please refer to Selective IgA deficiency guideline. Patients by definition have normal levels of IgG and IgM, and no paraprotein. Serum IgA levels do not reach adult levels until around 8 years of age. A diagnosis of IgA deficiency should not be made below the age of 4.

- **Common variable immune deficiency** is the most common significant primary antibody deficiency affecting children and adults. Patients have low IgG and IgA, with our without low IgM. There is often a delay of years between symptom onset and diagnosis. Such patients have infection as expected with antibody deficiency, but can also have autoimmune manifestations, especially cytopenias, as a result of immune dysregulation.

A large number of other rare primary antibody deficiency diseases are recognised, some resulting from confirmed genetic mutations affecting B cell development, with an inevitable impact on antibody production.

Patients with unexplained hypogammaglobulinaemia should be discussed with / referred to paediatric / adult immunology. Advice can be sought for adult patients via the NBT Immunology Advice and Guidance Service.

Referral should not be delayed for patients with symptomatic antibody deficiency.

Secondary Antibody deficiency

- This is much more common than primary. In nephrotic syndrome and protein losing enteropathy, the IgM level usually remains normal as IgM is retained as a result of it large size.
- The medication history should be reviewed as this may explain antibody deficiency e.g. certain anticonvulsants can be associated with hypogammaglobulinaemia, patients may have received B cell depleting therapy or other immune suppression. Secondary antibody deficiency is an ever expanding area, as increasing numbers of patients receive disease modifying, immune based therapies across many medical specialties

Causes of Secondary Hypogammaglobinaemia

Abnormal loss / increased catabolism	Conditions affecting Ig production
Nephrotic syndrome or other severe renal disease	Malnutrition or alcoholism
Severe burns	Drugs
Sepsis	Malignancy, especially haematological
Protein losing enteropathy	Rheumatological / connective tissue disease
Intestinal lymphangiectasia	Viral infection

Hypergammaglobulinaemia

The 2 main causes of hypergammaglobulinaemia are:

Increased monoclonal immunoglobulin (paraprotein) produced by a single clone of plasma cells, such as seen in monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma or other B cell lymphoproliferative disorders.

This results in an increase in 1 class of immunoglobulin (the paraprotein), which can be associated with suppression of the other 2 main classes, known as immune paresis. In this context, despite an increase in total immunoglobulin, patients can be

immunocompromised as the abnormal protein does not contribute to protective immunity.

- IgG and IgA paraproteins are more commonly associated with MGUS or myeloma, IgM paraproteins are more usually seen in low-grade lymphomas, however IgG and IgA paraproteins can be seen in low-grade lymphomas and occasionally IgM secreting myeloma is seen.

- Features suggesting clinically relevant paraproteins requiring haematology referral include CRAB:
    - Hyper**C**alcaemia
    - **R**enal impairment
    - **A**naemia
    - **B**one disease
    - Presence of lymphadenopathy, hepatosplenomegaly or constitutional symptom

- If MGUS is confirmed, monitoring should follow recommendations as per the laboratory report

Polyclonal increase in immunoglobulins, produced by many different plasma cells populations. This usually occurs in the context of immune stimulation associated with autoimmune or inflammatory disease and infection. Multiple Immunoglobulin classes are affected. Important causes of polyclonal hypergammaglobulinaemia include:

- Liver disease – cirrhosis, chronic hepatitis
- Connective tissue disease
- Chronic infection e.g. HIV, osteomyelitis
- Neoplasia

Elevate IgA in isolation is common and non-specific, being seen in pulmonary and gastrointestinal inflammatory diseases, some autoimmune conditions such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), liver disease, including alcohol consumptions and as above, it is also common in the elderly.

Referral of patients with polyclonal hypergammaglobulinaemia should be guided by the underlying cause e.g. to hepatology, rheumatology, haematology, infectious diseases.

Additional tests to consider:

- FBC
- LFT's
- HCV serology
- HIV testing
- CRP
- HEp2 assessment for underlying connective tissue disease

Repeat immunoglobulin assessment in these conditions should be guided by secondary care. In some conditions repeat testing is not required, whereas in other conditions, especially when immune suppressive therapy is indicated, monitoring for subsequent antibody deficiency is a consideration.

Causes of Hypergammaglobulinaemia

Polyclonal increase in any or all 3 Ig classes	**Monoclonal increase in 1 class, +/- decrease in the other 2 classes**
Liver disease – autoimmune hepatitis (high IgG) primary biliary cholangitis (high IgM), alcohol (high IgA)	MGUS
Connective tissue disease, e.g. SLE, RA, Sjogren's	Multiple myeloma (IgG, IgA, less common IgM)
Acute and chronic infection e.g. HIV, EBV, CMV	Chronic lymphocytic leukaemia
Haematological disorders	Non-Hodgkin lymphoma
Non-haematological malignancy	Waldentstrom's macroglobulnaemia (IgM)
	Primary systemic amyloidosis
	Monoclonal cryoglobulinaemias

References:

Lab Interpretation: Abnormal Immunoglobulin Levels in Adults. Ballow M. UpToDate February 2024. Literature review current through March 2024

Quantitative serum immunoglobulin tests. Loh R, Vale S, McLean-Tooke A. Aust Fam Physician 2013;**42**:195-198