



Abnormal laboratory results

Tests for cell-mediated immunity

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Summary

Patients with cell-mediated immunodeficiency experience recurrent infections with a broad range of pathogens, and an accompanying humoral immunodeficiency is not uncommon. A persistently low lymphocyte differential on a full blood count may provide a clue and should prompt further testing with quantification of lymphocyte subsets. Measurement of total immunoglobulins is a first-line screening investigation in suspected humoral immunodeficiency. Further investigations, which provide an *in vitro* or *in vivo* functional assessment, are highly specialised assays which are difficult to perform and interpret. Consultation with a specialist immunologist and the diagnostic laboratory is recommended.

Key words: hypogammaglobulinaemia, immunodeficiency.

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Introduction

Defence against potentially harmful pathogens is achieved by physical barriers such as skin and mucous membranes, and the coordinated efforts of the innate and adaptive immune systems. Innate immune responses are carried out by macrophages, neutrophils and natural killer cells, together with cytokines, complement and acute phase reactants such as C-reactive protein. Adaptive immunity relies upon B and T lymphocytes which express antigen-specific surface receptors. It can be divided into humoral (antibody-mediated and dependent upon B lymphocytes) and cellular (coordinated by T lymphocytes) immunity. While this distinction is oversimplified and somewhat inaccurate in that both types of responses are dependent upon helper T lymphocytes, it provides a useful model for classifying and evaluating suspected immunodeficiency.

Immunodeficiency

This occurs when failure of any part of the immune system leads to an increased predisposition to infection and associated sequelae such as autoimmunity and malignancy. Primary immunodeficiency results from genetic mutations of components

intrinsic to the immune system. Clinical diagnosis should be accompanied by molecular identification of a genetic mutation wherever possible to confirm the diagnosis, identify genotype-phenotype correlation, assist with genetic counselling and identify suitable candidates for gene-specific therapy. Secondary immunodeficiency results from defective immune function as a consequence of another condition such as HIV infection. Drugs such as corticosteroids, azathioprine, methotrexate or cyclosporin can also cause secondary immunodeficiency. Subtle impairment of immune function can also accompany certain chronic medical conditions including diabetes and chronic renal failure.

Immunodeficiency can be classified functionally into humoral or cell-mediated arms, as dysfunction of either pathway is characterised by specific clinical presentations (Table 1). Possible investigations for suspected immunodeficiency are presented in Table 2. These tests should be performed when the patient is clinically well, and not during an acute infective illness.

Humoral immunodeficiency

Antibody deficiency, or hypogammaglobulinaemia, can occur as a result of intrinsic defects of humoral immunity (primary), or secondary to another pathological condition. It is the most common manifestation of primary immunodeficiency and encompasses a broad range of clinical diagnoses. Clinical presentation can range from asymptomatic, to recurrent, atypical or life-threatening infections. Encapsulated bacteria, such as *Streptococcus pneumoniae*, *Neisseriae* species and *Haemophilus influenzae*, pose a particular threat as well as other bacterial species including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Campylobacter fetus* and *Mycoplasma* species. Recurrent or unusually severe sinopulmonary infection, other infections (gastrointestinal, skin, joint or central nervous system), or evidence of end-organ damage such as bronchiectasis, should alert the doctor to the possibility of an underlying humoral immunodeficiency.

Measuring humoral immunity

The simplest initial investigation for this condition is to quantify immunoglobulin (Ig) concentrations (IgG, IgA and IgM). Normal levels, however, do not exclude a humoral defect and if clinical suspicion is high, then more advanced investigations can be undertaken. This includes measuring antibodies to specific

Table 1

Characteristic clinical presentations of immunodeficiency

Type of immunodeficiency	Clinical presentation
Humoral immunodeficiency	
Hypogammaglobulinaemia	Recurrent sinopulmonary infection: - <i>Streptococcus pneumoniae</i> - <i>Haemophilus influenzae</i> - <i>Neisseria</i> species Other bacterial infections such as gastrointestinal, central nervous system, joint Evidence of end-organ damage such as bronchiectasis, conductive hearing loss
Cellular immunodeficiency	
T cell dysfunction	Infections with: - intracellular bacteria (mycobacteria, salmonella) - viruses (Epstein Barr, cytomegalovirus, varicella zoster, herpes simplex) - fungi (candida, aspergillus, cryptococcus, histoplasma, pneumocystis) - protozoa (toxoplasma, microsporidium, cryptosporidium)
Interleukin-12 interferon gamma axis deficiency	Atypical mycobacterial and salmonella infections
Impaired response to <i>Candida</i> species	Persistent mucocutaneous candidiasis Autoimmune endocrinopathy
Combined immunodeficiency	
Combined T and B cell dysfunction	Combined features of T cell deficiency and hypogammaglobulinaemia
Severe combined immunodeficiency syndromes	Failure to thrive in children Opportunistic infection Overwhelming sepsis
Wiskott-Aldrich syndrome	Thrombocytopenia Eczema Infection with encapsulated bacteria
Ataxia telangiectasia	Sinopulmonary disease Cerebellar ataxia Oculocutaneous telangiectasia
DiGeorge syndrome	Hypocalcaemia Recurrent infection Cardiac disease Abnormal facial features
Hyper IgM syndromes	Recurrent pyogenic infection Opportunistic infection
Natural killer cell dysfunction	Recurrent herpes virus infection Recurrent papillomavirus infection (warts)
Phagocyte defects	Recurrent pyogenic infections Recurrent abscesses
Ig immunoglobulin	

antigens following vaccination to assess if the patient produces a functional antibody response. This is usually performed in conjunction with assessment by a clinical immunologist. Immunoglobulin G subclasses can also be quantified – however the clinical utility of this investigation is somewhat controversial.

Cell-mediated immunodeficiency

Defective T cell-mediated immunity predisposes patients to a broader range of infections than humoral immunodeficiency, including intracellular pathogens, persistent superficial candidiasis or recurrent viral, fungal or protozoal infections

(Table 1). Defects can again be classified as either primary, or secondary to extrinsic factors. HIV infection resulting in progressive depletion of CD4 T cells is a particular consideration. As helper T cells are required for B cell-mediated antibody production, T cell immunodeficiency can result in functional B cell defects, thus patients with cell-mediated immunodeficiency often have an accompanying hypogammaglobulinaemia. This is termed combined immunodeficiency.

Measuring cellular immunity

Measurement of cell-mediated immunity can be undertaken by both *in vitro* and *in vivo* methods. It is, however, more problematic than humoral assessment as assays are plagued by difficulties in standardisation, biological variability, imprecision and technical complexity. Most tests are highly specialised and referral to a clinical immunologist is recommended.

Flow cytometry

The first step in the evaluation of cell-mediated immunity is to quantify circulating immune cells and their subsets by flow cytometric analysis. Patients' blood cells are incubated with fluorochrome-labelled monoclonal antibodies directed against cell surface molecules and analysed by a flow cytometer, which measures light scatter and fluorescence emission from individual cells. Different cell populations (B cells, and CD4/CD8 T cells and natural killer cells) can be distinguished based on their scatter profile and surface molecule expression. Absolute cell numbers are calculated as a percentage of the total white cell count and results are compared to age-matched reference ranges. It is important to note, however, that analogous to immunoglobulin measurement, quantification of lymphocyte numbers does not give an indication of their functional capacity. Lymphocyte subset analysis aids in the diagnosis and classification of paediatric severe combined immunodeficiency syndromes, and is also recommended in the evaluation of hypogammaglobulinaemia in common variable immunodeficiency. Quantifying CD4 T lymphocytes provides prognostic information and gives an indication of susceptibility to opportunistic infections in patients with HIV infection.

Delayed-type hypersensitivity skin testing

Delayed-type hypersensitivity skin testing provides a functional *in vivo* assessment of cellular immunity. The skin response following intradermal inoculation of antigen is dependent on antigen-specific memory T cells and results in local inflammation after 48–72 hours due to the recruitment of mononuclear cells (lymphocytes, monocytes) and neutrophils. By convention, a diameter of 5 mm induration is accepted as a positive result. The most widespread use of this type of test is the Mantoux test, which assesses previous exposure to *Mycobacterium tuberculosis* or Bacillus Calmette-Guérin (BCG) vaccination by evaluating the skin response to intradermal tuberculin. Other ubiquitous

Table 2

Investigations for suspected immunodeficiency

Suspected immunodeficiency	Screening tests	Advanced investigations
Humoral immunodeficiency	IgG, IgA, IgM Full blood count and differential* Lymphocyte subsets*	IgG subclasses Specific antibody titres and response to vaccination
Cellular immunodeficiency	Full blood count and differential* Lymphocyte subsets* HIV testing if indicated IgG, IgA, IgM	Delayed-type hypersensitivity skin tests Lymphocyte proliferation assays Natural killer cell cytotoxicity

Ig immunoglobulin

* defer testing until resolution of acute infective illness

antigens that can be tested include tetanus, candida and certain bacterial antigens. Skin responses are dependent upon previous exposure to the antigen and thus this test is of little use in infants less than six months of age.

Skin testing identifies functional memory T cells to a particular antigen, or the presence of cutaneous anergy. The latter is defined as an impaired cutaneous hypersensitivity response to a panel of common antigens and is consistent with cellular immune dysfunction. Causes of cutaneous anergy are listed in Table 3.

Lymphocyte proliferation assays

Lymphocyte proliferation assays are indicated if there is a suspicion of a defective cellular immune response either globally or to a specific antigen such as candida. The patient's peripheral blood mononuclear cells are incubated *in vitro* for 3–5 days with either a mitogen (substance which induces cellular division) or a recall antigen (to which the patient has been previously exposed). Radioactive thymidine is added to the culture and subsequently incorporated into the DNA of dividing cells. Radioactivity of the cell culture is measured after 24 hours and is directly proportional to the degree of induced cellular proliferation. Peripheral blood mononuclear cells from a healthy control are evaluated in parallel for comparison.

These assays are technically complex and are only performed by specialist laboratories. As the investigation can be time-consuming, it is advisable to first discuss the appropriateness of testing and choice of assay with the laboratory. Results are affected by immunosuppressive drugs, severe nutritional deficiencies and intercurrent illness¹ and these factors must be considered when interpreting results. As with skin testing, the patient must have been previously exposed to the antigen, thus antigen proliferation assays are not feasible in babies less

Table 3

Causes of cutaneous anergy*

Cause	Examples
Drugs	Corticosteroids (usually high dose) Immunosuppressants Chemotherapy
Immunodeficiency	Ataxia telangiectasia Severe combined immunodeficiency syndrome DiGeorge syndrome Wiskott-Aldrich syndrome
Infection	HIV Influenza Measles Mumps Active tuberculosis
Other conditions	Malignancy Chronic lymphocytic leukaemia Sarcoidosis Chronic renal failure Chronic liver disease
Testing errors	Poor technique Inadequate antigen dose

* impaired skin response to antigen

than six months of age. Response to mitogens, however, can be performed at any age from birth onwards.²

Other assays measuring lymphocyte activation

Other functional *in vitro* measures of lymphocyte activation include determining changes in surface marker expression (CD25, CD69, CD71) following activation³ or measurement of intracellular cytokines of T lymphocytes.

T cell proliferation following stimulation can be measured by succinimidyl ester of carboxyfluorescein diacetate (CFSE) dilution techniques,⁴ or T cell cytokine production quantified by ELISPOT assays. These assays, however, are not in routine use and are confined to research or specialised reference immunology laboratories.

The recently introduced interferon gamma (IFN γ) release assays measure T lymphocyte production of IFN γ in response to antigen exposure thereby providing an assessment of cell-mediated immunity. As with delayed-type hypersensitivity skin testing, clinical application is currently confined to the domain of tuberculosis latency and exposure.

Natural killer cell cytotoxicity assays

Assessing natural killer cells is indicated in patients suffering recurrent infection with herpes virus, or papillomavirus (associated with cutaneous warts). Natural killer cell cytotoxicity is assessed

by a ⁵¹Cr-release assay in which patients' natural killer cells are incubated with ⁵¹Cr-labelled target cells. Lysis of the target cells by natural killer cells leads to the release of radioactivity which can be measured. Natural killer cell dysfunction may occur in patients with CD16 genetic mutations, chronic mucocutaneous candidiasis, severe combined immunodeficiency and other cellular immunodeficiency syndromes.⁵ These conditions need to be considered and excluded if natural killer cell dysfunction is confirmed. As with T and B lymphocytes, functional natural killer cell deficits can occur even when natural killer cell counts are normal. Natural killer cell assays are technically complex and are rarely performed in diagnostic immunology laboratories.


Conclusion

The evaluation of suspected immunodeficiency is guided by clinical presentation. Screening tests of humoral and cellular immune function are initially performed, followed by referral to a specialist for more advanced investigations if clinically indicated (Table 2). Secondary causes of immunodeficiency, including HIV infection, need to be considered and excluded. When interpreting results, confounding factors such as immunosuppressive drug therapy and patient comorbidities, as well as analytical variables such as assay precision and reproducibility, need to be considered.

References

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Conflict of interest: none declared

Self-test questions 

The following statements are either true or false (answers on page 95)

1. Persistent superficial candidiasis may be a sign of T cell dysfunction.
2. Normal immunoglobulin concentrations exclude a humoral immunodeficiency.