Primary immunodeficiency diseases

'Look deeper into nature, and then you will understand everything better.' – ***Albert Einstein***

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Suvarna Buldeo has a special interest in the diagnosis of immunological conditions, especially primary immunodeficiency diseases. She attended the Basic and Advanced Courses in Clinical Immunology hosted by the Federation of Clinical Immunology Societies, USA, and completed a fellowship in Clinical Immunology at the University of Utah. She hopes to use the knowledge gained to improve immunology diagnostic services in South Africa.

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Melinda Suchard has an interest in flow cytometry, particularly its application to infectious disease immunology and immuno-regulation. She is currently registered for a PhD, the aim of which is development of a novel method to enumerate antigen-specific T lymphocytes. Other research interests include detection of regulatory T cells and indoleamine 2,3 dioxygenase. Dr Suchard runs a diagnostic flow cytometry laboratory for kidney transplant immunology. She co-ordinates a Short Course in Basic and Advanced Immunology for clinicians and scientists at Wits and hopes to see immuno-pathology become an HPCSA-accredited medical specialty.

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Primary immunodeficiency diseases (PIDs) are a group of disorders with defects in the development or function (or both) of the immune system. Most PIDs originate from mutations in single genes, but polygenic forms do occur.[1] The global prevalence of PIDs varies between 0.3 and 12 per 100 000 population, and is higher in areas with high rates of consanguinity.[2] The prevalence in South Africa is unknown, but according to prevalence data reported from the PID register,[3] these diseases are either missed or not reported. The possible reasons for under-diagnosis are that patients presenting with recurrent, persistent, severe or even unusual infections are treated without investigating the underlying cause, or the diagnosis is missed in the face of the overwhelming burden of similar clinical presentations of infectious diseases such as HIV and tuberculosis.

Early diagnosis is important as therapeutic options are available to treat and prevent long-term sequelae such as bronchiectasis, which will improve quality of life and decrease mortality.[4] Basic laboratory assays to screen for PIDs are available throughout South Africa but the threshold for investigation among healthcare workers is high. This review aims to increase the clinical suspicion of PIDs in South Africa and provide an approach to the diagnosis and management.

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Warning signs

PID should be strongly suspected in an individual who has a family history of the disease. Warning signs are failure to thrive or a history of infections that are **S**evere (requiring intravenous antibiotics and hospital admission), **P**ersistent (difficult to treat with standard regimens), caused by **U**nusual infective organisms (opportunistic pathogens) and **R**ecurrent (repeated infection at the same site or with the same organism). These infective features have been given the acronym SPUR. PID should also be suspected in individuals with early-onset autoimmune diseases or malignancy.[5]

Presentation may occur soon after birth or may be delayed, depending on the immune defect.[1] Typically, T lymphocyte and combined defects are severe and patients present before 6 months of age. Patients with antibody deficiencies generally present after 6 months of age when passive maternal antibodies have declined, or often much later in life. Patients with complement and phagocytic defects frequently have a delayed presentation.[6]

Major immune defects and their associated infections[1,7]

The immune system comprises the innate (complement and phagocytes) and adaptive responses (T and B lymphocytes). The

Table 1. Infections commonly associated with the major immune defects

Immune defect	Bacteria	Virus	Fungi	Protozoa
Complement deficiency	*Neisseria* spp., streptococci, *Haemophilus influenzae,* other encapsulated bacteria	-	-	-
Phagocytic defects	*Staphylococcus aureus, Pseudomonas aeruginosa*, non-tuberculous mycobacteria	-	*Candida* spp., *Aspergillus* spp.	-
Antibody deficiency	Streptococci, staphylococci, *Haemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa, Mycoplasma pneumoniae*	Enteroviruses	-	*Giardia lamblia*
T cell defects	Similar to antibody deficiencies but also includes the intracellular bacteria *Salmonella typhi, Listeria monocytogenes* and non-tuberculous mycobacteria	All viruses	*Candida* spp., *Aspergillus* spp., *Cryptococcus neoformans, Histoplasma capsulatum*	*Pneumocystis jirovecii, Toxoplasma gondii, Cryptosporidium parvum*

Modified from referance 10.

Table 2. Classification of PIDs

PID group	Associated features	Example
1. Combined immunodeficiencies	Failure to clear live vaccines, early-onset infections (*Pneumocystis jirovecii,* viral or bacterial), persistent *Candida* infections	Severe combined immunodeficiencies (SCIDs)
2. Well-defined syndromes with immunodeficiency	Distinct clinical features in addition to immune deficiency	DiGeorge anomaly
3. Predominantly antibody deficiency	Decreased antibody levels with recurrent infections, especially sinopulmonary (encapsulated bacteria) and gastrointestinal infections (enterovirus and *Giardia*)	Severe reduction in at least two serum immunoglobulin isotypes with normal or low numbers of B cells, e.g. combined variable immunodeficiency disorders (CVIDs)
4. Diseases of immune dysregulation	Significant autoimmune manifestations	X-linked lymphoproliferative disorders
5. Congenital defects of phagocyte number, function, or both	Recurrent and severe bacterial, mycobacterial and fungal infections (respiratory, cutaneous) or deep-seated abscesses	X-linked chronic granulomatous disease
6. Defects in innate immunity	Recurrent bacterial, viral (in particular herpes simplex), and fungal (often *Candida*) infections	Chronic mucocutaneous candidiasis
7. Auto-inflammatory disorders	Recurrent fever, rash, urticaria	Defects affecting the inflammasome, e.g. hyper-IgD syndrome
8. Complement deficiencies	Recurrent *Neisseria* spp. infections	Late complement component deficiency

complement system recruits phagocytes, enhances phagocytosis or directly lyses bacteria. Phagocytes (neutrophils, monocytes, macrophages and dendritic cells) engulf extracellular pathogens and destroy them by intracellular killing mechanisms. If phagocytes cannot kill the pathogen, foreign pathogen peptides are presented to T lymphocytes. Intracellular pathogens (e.g. viruses) activate CD8+ cytotoxic T lymphocytes to kill the infected cell. Extracellular pathogens (e.g. most bacteria) activate CD4+ T helper lymphocytes, which help B cells to produce antibody. CD4+ T lymphocyte defects therefore result in a picture of both T and B cell dysfunction – a

combined deficiency similar to the features seen in HIV infection.

Infections commonly associated with the major immune defects are given in Table 1. The type of infection can assist the clinician in recognising particular syndromes and in directing the laboratory investigations.

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Classification of PID

The Expert Committee of the International Union of Immunological Societies updated the classification of PIDs in 2011.[8] PIDs can be classified into 8 major groups (Table 2).

Differential diagnosis[6]

The differential diagnosis includes causes of secondary immune deficiency such as chronic infections, malnutrition, use of immunosuppressive drugs, splenectomy, malignancy and burns.

Diagnosis

The most critical part of the medical evaluation is obtaining a good history and performing a physical examination, as the site and types of infections may provide clues about the underlying defect.

Laboratory investigations and their clinical significance

A stepwise approach should be employed when investigating a patient for a possible PID.[7]

Step 1

- Exclude causes for secondary immune defects, e.g. HIV or *Mycobacterium tuberculosis* infection, or do a sweat test to exclude cystic fibrosis.
- Full blood and differential count with peripheral smear: This is used to assess the white cell counts and to identify specific morphological features. The following features may be relevant to PID:
  - **Neutropenia** – may be congenital, cyclic or occur in aplastic anaemia.
  - **Lymphopenia** – suggests a T cell disorder because approximately 70% of circulating lymphocytes are T cells.
  - **Leukocytosis** that persists between infections may occur in leukocyte adhesion deficiency (LAD).
  - **Thrombocytopenia** and the presence of microthrombocytes in male infants suggest Wiskott-Aldrich syndrome.
  - **Anaemia** may suggest autoimmune haemolytic anaemia, which may occur in common variable immune deficiency (CVID), or anaemia of chronic disease.
  - **A peripheral blood smear** should be examined for features of asplenia or impaired splenic function. Granulocytes may have morphological abnormalities (e.g. giant granules in Chédiak-Higashi syndrome).
  - **Absent staining of myeloid cell granules shows** myeloperoxidase deficiency.
- Quantitative immunoglobulins for immunoglobulin M (IgM), IgG, IgA and IgE. IgD is not routinely measured. Antibody deficiencies account for the majority of reported cases of PID. Selective IgA is the most common PID, and the majority of these patients are asymptomatic. IgA is absent in selective IgA deficiency. Low levels of immunoglobulins with normal B lymphocyte numbers suggest CVID. High IgM levels with low IgG/IgA/IgE occur in class switch recombination deficiencies. High IgE levels are characteristic of the hyper IgE syndrome but also found in Omenn syndrome, which is a combined immune deficiency.
- Complement levels are evaluated by the CH50 and AH50 – screening tests that assess the ability of the classic and alternative complement pathways respectively to haemolyse red blood cells. Reduced haemolytic activity is noted in complement factor deficiencies, but also during infections or immune complex diseases. Alternatively, complement factors can be directly measured.
- Quantitation of lymphocytes, which is the enumeration of B lymphocytes, T lymphocytes and natural killer (NK) cells by flow cytometry. Absence of B cells with normal T cells and NK cells is in keeping with agammaglobulinaemia, while reduced T cells are found in severe combined immunodeficiency syndrome (SCID).

These simple tests are usually sufficient to make the diagnosis of a PID and to commence therapy or refer the patient. If the above-mentioned tests are normal, and suspicion of PID remains, functional assessments (step 2) should be undertaken.

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Step 2

- Functional analysis of B lymphocyte and IgG subclasses. Analyse antibody responses to protein antigens (e.g. antibody response to tetanus vaccine) and polysaccharide antigens (e.g. antibody response to *Streptococcus pneumoniae* polysaccharide vaccine). If the antibody response to a vaccine antigen is low, the patient should be vaccinated to ensure appropriate exposure, and antibody levels should be retested 4 weeks after vaccination. IgG subclasses should only be requested if total IgG is normal, if IgA is absent, hyper IgE syndrome is suspected or antibody responses to vaccines are poor.
- Functional analysis of T lymphocytes. T lymphocyte function can be measured by analysing cytokine production

or proliferation. The Mantoux test is an *in vivo* delayed hypersensitivity response to purified peptide derivative. T lymphocytes proliferate in response to antigens and mitogens. Tests for function require viable cells and must reach the laboratory within 6 - 12 hours.

- Functional analysis of the innate immune system, including phagocytic defects. Chemotaxis (ability of neutrophils to migrate to the site of infection), phagocytosis (engulfment of foreign pathogens), and the oxidative burst (intracellular killing) functions of neutrophils and monocytes can be assessed.

Step 3

Molecular tests are performed to confirm the diagnosis of specific defects. This will be guided by results of tests performed in steps 1 and 2. For example, identification of a mutation in Bruton's tyrosine kinase gene is used to confirm the diagnosis of X-linked agammaglobulinaemia.

Neonatal screening for severe primary immunodeficiency

Severe combined immunodeficiency is a medical emergency requiring urgent bone marrow transplantation and avoidance of live vaccines, such as the oral polio and bacille Calmette-Guérin (BCG), routinely given at birth. The total lymphocyte count may be used to screen for severe combined immunodeficiency. While routine screening is not practised in South Africa, it should be essential for any child born to a mother who has previously lost an infant owing to an infectious cause. Neonatal screening for T lymphopenia by molecular methods from dried blood spots is available in some areas of the USA.[9]

General principles of management[1]

Supportive

- Prevent infections with prophylactic antibiotics and/or antifungals. The choice of antimicrobial will depend on the immune defect. Penicillin is commonly used for late complement factor deficiencies, co-trimoxazole and itraconazole for chronic granulomatous disease and co-trimoxazole for T cell defects.
- Treat infections promptly and aggressively. Antibiotics should be prescribed in accordance with microbiological culture and drug sensitivity testing.
- Give nutritional support, as micro nutrients such as zinc, vitamin D and vitamin A are required for immune function.
- Use only irradiated blood products for transfusions to prevent possible graft versus host disease.
- Avoid the use of live attenuated vaccines, e.g. oral polio, BCG, measles, mumps, rubella, varicella or rotavirus, in any patient with T cell or severe immunodeficiency to prevent vaccine-associated infections. Live vaccines should be avoided whenever there is a family history of severe PID.
- Do a chest X-ray to exclude the presence of chronic lung disease.
- Notify patients on the South African PID register.
- Refer the patient for specific treatment. The laboratory can assist with referral details.

Specific

- B lymphocyte and antibody deficiencies: Lifelong intravenous or subcutaneous immunoglobulin supplementation.
- SCID: Haematopoietic stem cell transplant from an HLA identical sibling. Gene therapy has been shown to be successful, as immune reconstitution was achieved in children with SCID. However, it is not yet routinely used as a therapeutic modality as it caused lymphoma in some recipients.
- Phagocytic defects: Interferon gamma supplementation.

In a nutshell

- PIDs are caused by genetic defects in the immune system.
- Suspect PID in any individual with SPUR (**S**evere, **P**ersistent, **U**nusual and **R**ecurrent infections).
- PIDs cause severe morbidity and irreversible infection-related pathology, which can be prevented by early diagnosis.
- Simple screening assays to assist with diagnosis are available. Screening tests include a full blood count and differential, enumeration of B, T and natural killer (NK) cells, quantitation of immunoglobulins and measurement of complement components.
- Laboratory work-up should be guided by the history and examination.
- Appropriate therapeutic options to treat, prevent organ pathology and improve quality of life are available.
- Antibody deficiencies are the most common PIDs and are easily treated with immunoglobulin supplementation.
- Avoid live vaccines in patients with severe PIDs.
- Treat infections aggressively and use prophylactic antimicrobials to prevent new infections.

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