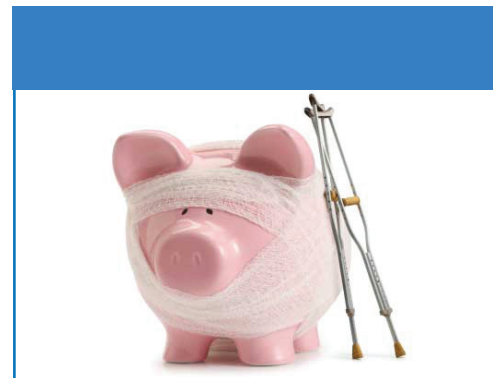


PATHCHAT

by Dr Sylvia van den Berg and Dr Cathy van Rooyen

Endorsed by the Infectious Diseases Peer Group

Primary immunodeficiencies: A diagnostic approach



Primary immunodeficiencies are a group of heterogenous genetic disorders that cause an enhanced susceptibility to recurrent and severe infections.

Features that suggest a primary immunodeficiency:

(First three features most predictive)

- Family history of immunodeficiency or unexplained early death (<30 years)
- Failure to thrive
- Need for IV antibiotics and/or hospitalisation to clear infection
- Six or more new infections in one year
- Two or more sinus infections or pneumonia in one year
- Four or more new ear infections in one year
- Two or more episodes of sepsis or meningitis in a lifetime
- Two or more months of antibiotics with little or without an effect
- Recurrent or resistant *Candida* infections
- Recurrent tissue or organ abscesses
- Infection with an opportunistic pathogen
- Structural damage due to infections
- Complications from a live vaccine
- Chronic diarrhoea
- Non-healing wounds
- Extensive skin lesions
- Persistent lymphopaenia
- Unexplained fever or autoimmunity

Additional features in infants include the following:

- Delayed umbilical separation (>30 days)
- Congenital heart defects
- Hypocalcaemia

- Absent thymic shadow on CXR

Most children referred for recurrent infections do not have an underlying immunodeficiency. The majority of children will have increased exposure, allergy (~30%), other chronic diseases (~10%) or an anatomic defect. Only about 10% will have an immunodeficiency.

Infections with certain sentinel organisms should set off the alarm bells and include the following:

- Recurrent sinopulmonary infections with encapsulated bacteria (*S. pneumoniae*, *H. influenzae* type b, *M. catarrhalis*)
- *Pneumocystis jirovecii* pneumonia
- *Pseudomonas aeruginosa* infections
- Enteroviral meningoencephalitis
- *Cryptosporidium* enteric infections
- Recurrent infections with *S. aureus*, coagulase negative staphylococci, *Serratia marcescens*, *Chromobacterium violaceum* or *Aspergillus* spp.
- Recurrent herpes viral infections
- Infections with live vaccines (including BCG, oral polio vaccine, measles, rota virus, varicella)
- Prolonged or recurrent *Candida* infections
- Recurrent invasive neisserial infections
- Systemic or deep infections with nontuberculous mycobacteria

For more information or clinical queries, contact:

Dr Cathy van Rooyen

Tel: 012 678 0613/6

Email: vanrooyenc@ampath.co.za

Dr Sylvia van den Berg

Tel: 012 678 0613/7

Email: vandenbergs@ampath.co.za

Major subtypes of primary immunodeficiencies* and expected clinical findings:

Subtype:	Age at onset:	Pathogens:	Infections:
Humoral immunodeficiencies: X-linked or autosomal recessive agammaglobulinemia CVID IgG subclass deficiency IgA with IgG subclass deficiency Selective IgA deficiency Specific antibody deficiency with normal immunoglobulins Transient hypogammaglobulinemia of infancy	>6 months; can present in adulthood; CVID can present in adolescence	Encapsulated bacteria Fungi and parasites <i>Giardia lamblia</i> <i>Cryptosporidium</i> species Enterovirus	Sinus infections Otitis media Bronchiectasis Chronic conjunctivitis Pyoderma Enteroviral infections Infectious diarrhoea
Combined immunodeficiencies: T-B+ SCID T-B- SCID Omenn Syndrome CD40 ligand deficiency ZAP-70 deficiency MHC class I deficiency MHC class II deficiency Complete DiGeorge Syndrome DOCK8 deficiency	<6 months	Mycobacterium species Viruses: Cytomegalovirus Epstein-Barr Varicella virus Enterovirus, etc. Candida <i>Pneumocystis jirovecii</i>	Opportunistic infections Severe recurrent bacterial and viral infections Oral thrush Excessive diarrhoea
Innate immunodeficiencies: NK cell deficiency AIRE deficiency NEMO deficiency Herpes simplex encephalitis Chronic mucocutaneous candidiasis WHIM	Childhood	Mycobacteria and other pyogenes <i>Candida albicans</i> <i>Histoplasma capsulatum</i> <i>Aspergillus fumigatus</i> <i>Coccidioides immitis</i> Herpes viral infection Human papilloma virus	Recurrent sinopulmonary infections with encapsulated bacteria <i>Mucocutaneous candidiasis</i> Herpes viral infections
Phagocytic disorders: Chronic granulomatous disease Leukocyte adhesion deficiencies Defects of neutrophil differentiation MSMD	Infancy or childhood	<i>S. aureus</i> <i>Pseudomonas</i> <i>S. marcescens</i> <i>Nocardia</i> <i>Klebsiella</i> spp <i>Salmonella</i> serovar typhi Mycobacteria BCG <i>Candida aspergillus</i>	Skin, oral cavity and anorectal infections Poor wound healing Abscesses
Complement deficiencies: C1-9 deficiencies Mannan binding lectin deficiency Factor D, I and H deficiencies	Any age	Neisserial infections <i>S. pneumoniae</i> <i>H. influenzae</i> type b Mycobacteria Legionella	Pyogenic infections with encapsulated organisms Meningococcal and gonococcal infections Tuberculosis Legionnaire's disease Hemolytic-uraemic Syndrome
Well-defined syndromes with immunodeficiencies: Wiskott-Aldrich Syndrome Ataxia-telangiectasia DiGeorge anomaly Hyper-IgE syndromes Dyskeratosis congenita	Infancy or childhood		

*The major subtypes of immunodeficiencies are provided with some examples. A complete classification can be obtained from Al-Herz W, Bousfiha A, Casanova JL et al. Primary immunodeficiency diseases: An update on the classification. International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. *Frontiers in Immunology* 2011; 2:1–26.

(CVID: Common variable immunodeficiency; SCID: Severe combined immunodeficiency; MSMD: Mendelian susceptibility to mycobacterial disease; WHIM: Warts, hypogammaglobulinaemia, infections, myelokathexis)

Adapted from: Merritt TH, Segreti J. The role of the infectious disease specialist in the diagnosis and treatment of primary immunodeficiency disease. *Infectious diseases in clinical practice*, 2011;19:316–325 and Al-Herz W, Bousfiha A, Casanova JL et al. Primary immunodeficiency diseases: An update on the classification. International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. *Frontiers in Immunology* 2011; 2:1–26.

Investigations

Abnormal results need to be verified by a repeat investigation when the patient is well, as external factors like infections and medication may have a transient influence on the test results.

1. Test for HIV, CMV, EBV, TB, disseminated BCGosis, PCP and other infections where relevant
2. FBC and differential count
3. CRP and ESR
4. Chest X-ray: To determine the presence of a thymic shadow and to assess the presence of lung disease in children and adults
5. Screen for cystic fibrosis or allergies where indicated

1. Antibody (humoral) deficiencies – these account for about 70% of primary immunodeficiencies

Patients suffering from antibody deficiencies have a problem with recurrent, severe upper (recurrent pneumonia) and lower respiratory tract infections (i.e. recurrent otitis media, sinusitis), especially with pyogenic organisms, such as *S. pneumoniae* and *H. influenzae*.

Appropriate initial tests would be the following:

- Full blood count and differential
- KREC PCR – Neonatal screening on neonatal blood spot (NBS) or EDTA blood to exclude agammaglobulinaemia before administering live oral polio vaccine
- Quantitative immunoglobulins **prior** to the administration of immuno-globulins: IgG, M, A and E
- IgG subclasses
- Specific antibody titres to *S. pneumoniae*, *H. influenzae*, tetanus and diphtheria. If specific antibody levels are low, booster immunisations should be administered and titres measured again three to four weeks later.

Secondline testing:

- Secretory IgA

Should any abnormality be detected, additional tests should be done:

- Lymphocyte immunophenotype: to determine total B-cell numbers
- KREC PCR in patients with agammaglobulinaemia
- B-cell function testing for B-cell activation markers
- Memory B-cells: flow cytometric assay to assess germinal B-cell function in a patient with a humoral immunodeficiency. Low memory B-cells are associated with more serious or frequent infections, autoimmune disease and granulomatous disease
- IL17: Decreased in Hyper-IgE syndrome

Diagnostic tests:

- Bruton's Tyrosine Kinase: flow cytometric assay in patients with suspected X-linked agammaglobulinaemia
- CD 40 Ligand: flow cytometric assay in patients with suspected hyper-IgM syndrome
- Genetic studies
- Flow cytometric markers of B-cell maturation and subtypes of CVID will be available in the near future. Please contact the laboratory for further information.

2. T-cell defects – these account for about 15% of primary immunodeficiencies

T-cell defects usually present at a very young age with life-threatening infections due to a wide range of different pathogens, often opportunistic infections. Infections are persistent and severe, and viruses, fungi and intracellular bacteria are often involved. A family history of unexplained, especially infective deaths, should prompt further investigation.

- HIV ELISA or PCR in babies: done in all patients suspected of having a T-cell deficiency
- FBC and differential: patients are usually lymphopaenic with persistent absolute lymphocyte counts $<1.5 \times 10^9$ in older children and $<2.5 \times 10^9/l$ in younger children
- TREC PCR: neonatal screening on NBS or EDTA blood to exclude SCID before administration of any live vaccines and for early diagnosis and search of bone marrow transplant
- Lymphocyte immunophenotyping to enumerate the lymphocyte subtypes (B-, T- and NK-cells)
- Lymphocyte proliferation tests to mitogens
 - PHA
 - PMA
 - PMA+ionophore
 - CD3
 - CD3 + IL-2
 - CON A
 - PWM
- Lymphocyte proliferation tests to recall antigens
 - Varicella zoster
 - Candida
 - Tetanus

For infants and young children, all of the lymphocyte proliferation tests to mitogens should be ordered, whereas only LPT to candida, varicella zoster and PHA should be ordered in adults.

- Specialised immunopenotyping:
 - Naive and memory CD4- and CD8-cells
 - Alpha/beta, gamma/delta T-cells
 - HLA DR
 - Common gamma chain
 - Interleukin 7 Receptor alpha
 - Test repertoire will be expanded in the near future. Please contact the laboratory for further information
- Genetic testing

3. Tests to determine neutrophil function

These tests should be requested in patients with recurrent skin, soft tissue or deep abscesses, or recurrent infections with *S. aureus*, coagulase negative staphylococci, *S. marcescens*, *P. aeruginosa*, *Chromobacterium violaceum* or *Aspergillus*.

- FBC with differential count
- Neutrophil oxidative burst, phagocytosis and chemotaxis
- Leukocyte adhesion studies: CD11 and CD18: in babies with delayed umbilical cord separation (>30 days) and patients with recurrent bacterial infections, mainly involving the skin and mucous membranes, periodontitis, absent pus formation and impaired wound healing

- Neutrophil antibodies: in suspected autoimmune mediated neutropaenia

4. Tests to determine complement function

The total haemolytic complement activity should be requested in patients with recurrent neisserial and pyogenic infections.

Low MBL levels may predispose patients to upper respiratory tract infections and they may have a higher risk for severe meningococcal or pneumococcal infections. They may also be at increased risk of developing tuberculosis and legionnaire's disease. An increased incidence of infectious disease is also seen in immunocompromised patients or immunological immature neonates and infants with concomitant MBL deficiency. Immunocompromised hosts include critically ill patients in the ICU, cystic fibrosis patients and patients receiving chemotherapy. Patients with concomitant MBL and humoral immunodeficiency also tend to have more severe and recurrent infections. It must be kept in mind that a wide range of MBL serum levels have been observed in apparently healthy voluntary blood donors and low levels are not necessarily clinically relevant.

Symptomatic patients with normal antibody and neutrophil tests should be further evaluated by complement function testing.

- Classic and alternate pathways (CH100 and ACH100)
- Complement 3 and 4 levels
- Mannan binding lectin (MBL)

5. Natural killer cells

Natural killer (NK) cells play a crucial role in the host defence against herpes virus infections, especially herpes simplex virus and varicella zoster virus reactivation and latency.

- Total NK cell numbers
- NK cell function
- NK cell cytotoxicity (available soon)

6. Neonatal screening

It is crucial to make a diagnosis of SCID and X-linked agammaglobulinaemia (XLA)/Bruton's disease as early as possible, before the patients receive live vaccines or present with infections,

as live vaccines and infections can be fatal, and also greatly diminish the success rate of the bone marrow transplant.

Neonatal screening assays are now available at Ampath to detect diseases hallmarked by the absence of T- or B-lymphocytes, classically seen in SCID and XLA/Bruton's disease.

Neonatal PID screening tests:

- T-cell receptor excision circles (TRECs) – absent in SCID patients
- Kappa-deleting receptor excision circles (KRECs) – absent in XLA patients

A dried bloodspot on a Guthrie card obtained after a heel prick or EDTA blood shortly after birth is required. The test can also be used later as supporting evidence to help make a diagnosis of SCID or XLA.

7. Tests for causes of secondary immunodeficiency

Secondary causes of immunodeficiencies should always be excluded and include HIV/AIDS and other viral infections nutritional deficiencies, malignancies, infections, immunosuppressive medication, diabetes mellitus, dialysis and uraemia, protein-losing conditions, liver disease, malnutrition, trauma and burns, ionising and ultraviolet radiation, toxic chemicals, pregnancy, old age and severe stress.

Conclusion

As immunodeficiencies may be complex and difficult to diagnose, it is recommended that advice be sought from immunologists, paediatricians, physicians, ENT surgeons and pulmonologists in the workup of difficult patients. It cannot be overemphasised that a high index of suspicion should always be maintained for possible immunodeficiencies, as untreated immunodeficiencies are life threatening, and the long-term prognosis depends on early diagnosis and intervention. Infections should be promptly recognised and aggressively treated with appropriate antibiotics. IVIG or SCIG should **not** be given unless urgently indicated until there is a thorough evaluation of the immune system, as these will interfere with antibody investigations for months and have potential side effects. Only once the investigations are complete should they be carefully considered.

Dear Doctor,

We would like to request your assistance for the PID Registry work of South Africa.

Primary immunodeficiencies (PIDs) are inborn errors of immunity resulting in susceptibility to severe, recurrent, atypical infections and the consequences of immune dysregulation. Within a context of epidemic infectious disease rates, PIDs are orphan diseases often unrecognised or associated with delayed time to diagnosis. National and international efforts are ongoing to increase awareness, improve case finding, and describe the prevalence of PID worldwide. Ethical consent to conduct this research work is maintained at the universities of Stellenbosch, Cape Town and the Witwatersrand.

A South African database of patients with clinical, laboratory-based or genetically confirmed PID diagnoses has been compiled in Excel format as a registry since 2008. Patients are assigned IUIS classification according to diagnosis. Both the names and diagnoses are coded and protected on a secured database. The data will also be entered anonymously on the African (ASID) Registry and for international surveys.

Local efforts in the Western Cape have increased awareness, diagnoses and reporting of PID since the Registry's inception. However, there remains

under-representation of case-reporting from other provinces, particularly in black populations.

We invite you to contact us for further information, if you have identified new patients with probable or confirmed PID diagnosis. We appreciate your time constraints and will assist you wherever possible. On the database, the patients will be reflected under the relevant referring doctor/institution.

Thank you very much for your assistance.

Kind regards

Dr Monika Esser

Head of Immunology Unit
NHLS Tygerberg,
Stellenbosch University

Ms Rina Nortje

Secretary PID Register
rina@sun.ac.za
Fax: 021 938 4005; Tel: 072 210 0206

