Protocol for diagnosing primary immunodeficiency

Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update


The clinical presentations of PID

- Recurrent ENT and airway infections
- Failure to thrive from early infancy
- Recurrent pyogenic infections
- Unusual infections or unusually severe course of infections
- Recurrent infections with the same type of pathogen
- Autoimmune or chronic inflammatory disease; lymphoproliferation
- Characteristic combinations of clinical features (eponymous syndromes)
- Angioedema
# Table 1. Symptoms and signs that could point to potential PID

## A. History

### 1. The hallmark of PID: Infection history

- Recurrent (probably) bacterial infections (more frequent than expected at the patient’s age)
- More than one severe infection (e.g. meningitis, osteomyelitis, pneumonia, sepsis)
- Infections that present atypically, are unusually severe or chronic or fail regular treatment (especially if i.v. antibiotics are needed)
- Abscess of internal organ
- Recurrent subcutaneous abscesses (especially in children)
- Prolonged or recurrent diarrhoea
- Any infection caused by an unexpected or opportunistic pathogen (e.g. pneumocystis)
- Severe or long-lasting warts, generalized mollusca contagiosa
- Extensive candidiasis, recurrent oral thrush in children >1 year
- Complications of vaccination (disseminated BCG or varicella infection, paralytic polio, rotavirus infection)

### 2. Remember the family history!

- PID in the family; familial occurrence of similar symptoms (affected males related by the female line, or another clear pattern of inheritance).
- Unexplained early infant deaths, deaths due to infection
- Consanguinity in the (grand) parents (known or suspected)
- Autoimmune disease or haematological malignancy in several family members

### 3. Other* (could point to PID, but may not)

- Aplasia or hypoplasia of thymus (X-ray)
- Angioedema
- Auto-immune disease (especially auto-immune cytopenias, SLE)
- Bleeding tendency
- Congenital cardiac anomalies (mainly conotruncal defects)
- Chronic diarrhoea, malabsorption, pancreatic insufficiency
- Delayed separation of umbilical cord (>4 weeks)
- Delayed shedding of primary teeth
- Developmental delay (progressive)
- Difficult-to-treat obstructive lung disease
- Eczema, dermatitis (severe, atypical)
- Failure to thrive (child) or wasting (adult)
- Graft-versus-host reaction after blood transfusion, or mother-to-child (infant) engraftment
- Granulomas
- Haemolysis
- Hypercalcaemic seizures
- Inflammatory bowel disease (atypical)
- Malignancy (mainly lymphoma)
- Non-allergic oedema
- Poor wound healing; scarring
- Recurrent fever
- Rib or other skeletal anomalies (X-ray)
- Thymoma
- Unexplained bronchiectasis, pneumatoceles, interstitial lung disease
- Vasculitis

## B. Physical examination

### Skin and appendages

- Abnormal hair or teeth • Eczema • Neonatal erythroderma • (Partial) albinism • Pale skin • Incontinentia pigmenti
- Nail dystrophy • Extensive warts or molluscae • Congenital alopecia • Vitiligo • Petechiae (early onset / chronic)
- Cold abscesses • Telangiectasia • Absence of sweating

### Oral cavity

- Gingivostomatitis (severe) • Periodontitis • Aphthae (recurrent) • Giant oral ulcers • Thrush • Dental crowding
- Conical incisors • Enamel hypoplasia • Persistent deciduous teeth

### Eyes

- Retinal lesions • Telangiectasia

### Lymphoid tissue

- Absence of lymph nodes and tonsils • Lymphadenopathy (excessive) • Asplenia • Organomegaly (liver, spleen)

### Neurological

- Ataxia • Microcephaly • Macrocephaly

### Other

- Angioedema (without urticaria) • Digital clubbing • Dysmorphism • Stunted growth or disproportional growth

## C. Baseline blood tests

### Haematology

- Granulocytopenia, lymphocytopenia, or neutrophilia • Eosinophilia • Giant or absent granules in phagocytes
- Howell-Jolly bodies • Thrombocytopenia • Small platelets • Anaemia (aplastic, haemolytic)

### Chemistry

- Hypocalcaemia • Hypofibrinogenaemia • Hypertriglyceridaemia • Hyperferritinaemia • Low CRP and other inflammatory parameters during infections

* In alphabetical order. BCG: bacille Calmette-Guérin; CRP: C-reactive protein; i.v.: intravenous; PID: primary immunodeficiency; SLE: systemic lupus erythematosus.
### Table 2. Pattern recognition gives direction to the diagnostic process.

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Encountered pathogens</th>
<th>Special features</th>
<th>Non-immunological differential diagnosis</th>
<th>Diagnostic protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent ENT and airway infections (including bronchiectasis).</td>
<td>Mainly extracellular bacteria such as <em>Streptococcus pneumoniae</em>, Moraxella catarrhalis</td>
<td>Bronchiectasis. Recurrent bronchitis in a non-smoker. Unexplained chronic cough.</td>
<td>Frequency, children: normal frequency of infection in infants (day-care, passive smoking), bronchial hyperreactivity, allergy, otitis, adenoidal hypertrophy, iron deficiency anaemia, gastro-esophageal reflux.</td>
<td>Go to protocol 1</td>
</tr>
<tr>
<td>Most patients do not have PID. Even if they do, it is seldom life-threatening in the short term (but may cause organ damage in the long term). Exclude more frequent non-immunological problems first, except in case of a positive family history. Perform immunological tests in case of bronchiectasis. If &gt;31 pneumonia occurs, or when ENT infections persist abnormally long.</td>
<td>Sometimes: <em>Staphylococcus aureus</em>, <em>Neisseria meningitidis</em>, group A <em>Streptococcus</em>, <em>Mycoplasma pneumoniae</em>, Uniploasma urealyticum; Campylobacter jejuni, Helicobacter pylori.</td>
<td>Diarrhoea due to <em>Giardia lamblia</em>.</td>
<td></td>
<td></td>
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<tr>
<td>Failure to thrive from early infancy (including intractable diarrhoea, severe eczema). Only a few of these children have PID, but delay in diagnosis and treatment by SCT greatly impairs survival. Perform immunological tests in parallel with tests for other causes of failure to thrive.</td>
<td>Mainly viruses (CMV, EBV, VZV, HSV, adenovirus, HPVB, HPV, molluscum contagiosum, RSV), fungi (superficial Candida, <em>Aspergillus</em>, <em>Cryptococcus</em>, <em>Histoplasma</em>, <em>Pneumocystis jiroveci/canis</em>), protozoa (<em>Toxoplasma</em>, <em>Microsporium</em>, <em>Cryptosporidium</em>) and intracellular bacteria such as <em>Mycobacterium</em> spp. and <em>Salmonella</em></td>
<td>Intractable diarrhoea with or without rectal bleeding. Unusual infections or unusually severe course of infections, opportunistic infections.</td>
<td>A variety of gastrointestinal, renal, cardiopulmonary, endocrine, neurological, metabolic and congenital causes. Malnourishment: Chronic lead poisoning. Perinatal infection. Severe malnourishment (see appropriate textbooks).</td>
<td>Go to protocol 2</td>
</tr>
<tr>
<td>Unusual infections or unusually severe course of infections (unexplained – periodic fever, see 6). An uncommon presentation of a common disease is more common than an uncommon disease (such as immunodeficiency). Perform immunological screening tests at an early stage, however, because underlying immunodeficiency may be life-threatening.</td>
<td>Mainly intracellular bacteria such as <em>Mycobacterium</em> spp. and <em>Salmonella</em>; viruses (CMV, EBV, VZV, HSV, JC, HPV), fungi (Candida, <em>Aspergillus</em>, <em>Cryptococcus</em>, <em>Histoplasma</em>, <em>Pneumocystis jiroveci/canis</em>) and protozoa (<em>Toxoplasma</em>, <em>Microsporium</em>, <em>Cryptosporidium</em>).</td>
<td>May present later in life. Early onset; association of multiple features; atypical resistance to treatments; opportunistic infections.</td>
<td>Virulent strain of pathogen, reduced general condition of patient leading to secondary immunodeficiency (malignancy, malnutrition, chronic disease). Immunosuppressive therapy: HIV.</td>
<td>Go to protocol 2</td>
</tr>
</tbody>
</table>
| Recurrent infections with the same type of pathogen. Many have PID, but the recurrent infections may be life-threatening. Screening is therefore warranted. | Intracellular bacteria such as *Salmonella*, *mycobacteria*, *Neisseria*, *Streptococcus* meningococci. Yeasts, fungi such as conidia Encapsulated bacteria such as *pneumococci*. Viruses. | Normally no other recurrent infectious problems. No delayed fever/raise in CRP: deficiency in NF-kB signalling. (IRAK4, NEMO-CD2, IκBα deficiency). Encapsulated bacterial sepsis: asplenia. Excessive warts: epidermodysplasia verruciformis, WHIM, DOCK18. Herpesviruses: NK-cell deficiency. X-linked lymphoproliferative syndrome. | Increased exposure, coincidence. Inadequate treatment of first infection. Anatomical defect (e.g. fistula). Colonization. Occult infection acting as reservoir (e.g. endocarditis, abscess). Asplenia. | Go to protocol 2 2b

<p>| Autoimmune or chronic inflammatory disease; lymphoproliferation. Most cases of autoimmune short term (but may cause chronic inflammatory disease, and lymphoproliferation are not associated with recurrent infections. If the combination occurs, or if the case presents atypically or at an unexpected age, immunodeficiency is more likely. | When combinations of clinical presentations are present, look there. Generally autoinflammatory disorders do not present serious infectious problems. Distinct combinations of clinical presentations are present, associated with particular syndromes and cancomitant infectious problems. Identify syndrome by clinical features. (See appropriate textbooks). | (See appropriate textbooks). | (See appropriate textbooks). | Start with protocol 1, 2 or 3 guided by predominant clinical presentation (1–3, see above). When in doubt perform a combination of the tests in steps 1 from all three protocols. |
| Characteristic combinations of clinical features (episomony of disease). Many primarily non-immunological syndromes show features of immunodeficiency. See suggestive symptoms and signs in Table 1. | Different syndromes are associated with particular forms of immunodeficiency and concomitant infectious problems. Identify syndrome by clinical features. (See appropriate textbooks for non-immunological syndrome characteristics). | Follow appropriate protocol guided by predominant clinical presentation (1–4, see above). Perform appropriate tests for the particular syndrome. When in doubt perform a combination of the tests in step 1 from all three protocols. | Follow appropriate protocol guided by predominant clinical presentation (1–4, see above). Perform appropriate tests for the particular syndrome. When in doubt perform a combination of the tests in step 1 from all three protocols. | Go to protocol 1 step 2b. |
| Angioedema | Related to triggering factors (e.g. stress, trauma, menses). Symptoms typically last &gt;24 h. Not reacting to epinephrine/antihistamine/corticosteroid treatment. May mimic acute abdomen. | Allergy, malignancy, autoimmunity, ACE-inhibitor therapy. Idiopathic. | Go to protocol 1 step 2b. | |</p>
<table>
<thead>
<tr>
<th>Step 1</th>
<th>Rule out severe antibody deficiency and neutropenia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Perform</strong></td>
<td>Blood count and differential (check platelet volume, absolute lymphocyte, neutrophil and eosinophil counts). IgG, IgA, and IgM. IgE.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 2a</th>
<th>Predominantly antibody deficiencies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypogammaglobulinaemia</strong></td>
<td>If not secondary to drugs, lymphoid malignancy, thymoma, immunoglobulin loss (urine, faeces), perform: booster responses (tetanus; unconjugated pneumococcal vaccine if &gt;2–3 years of age; a rise in titre 3–4 weeks after vaccination appropriate for age to above a defined level should be considered a positive response), consider: IgG-subclasses (when IgG&gt;4g/l) and M-proteins.</td>
</tr>
<tr>
<td><strong>Next step</strong></td>
<td>Go to step 4.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 2b</th>
<th>Predominantly antibody deficiencies or complement deficiencies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal results step 1</strong></td>
<td>When positive family history or problems persist, perform: booster responses, CH50 and AP50, consider: IgG-subclasses and M-proteins; MBL, asplenia. In case of angioedema: C1-inhibitor level, C4 during attack.</td>
</tr>
<tr>
<td><strong>Next step</strong></td>
<td>Normal results: Wait and see. Repeat total IgG, IgA, IgM, and IgG-subclasses after 1–2 years (6 months if &lt;1 year of age), and booster responses after 3–5 years. Consider step 3. Consider lymphocyte subpopulations (Table 4), consider protocol 3. Abnormal results: go to step 4.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 3</th>
<th>Other potential PIDs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal results steps 1 &amp; 2</strong></td>
<td>When symptoms or signs from Table 1 are present: consult an immunologist to determine a specific work-up. Other potential explanations for recurrent infections do not always automatically exclude PID.</td>
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</table>

<table>
<thead>
<tr>
<th>Step 4</th>
<th>Final diagnosis</th>
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</thead>
<tbody>
<tr>
<td><strong>Abnormal results step 1</strong></td>
<td>Agammaglobulinaemia: lymphocyte subpopulations (Table 4), consider lymphocyte proliferation tests (Table 4), B cell maturation analysis in bone marrow. Genetic determination of defect if possible.</td>
</tr>
<tr>
<td><strong>Abnormal results step 2</strong></td>
<td>IgG-subclass deficiency, IgA deficiency, abnormal booster responses, and/or hypogammaglobulinaemia: lymphocyte subpopulations (Table 4), consider lymphocyte proliferation tests (Table 4), chromosomal analysis, α-fetoprotein. Genetic determination of defect if possible. If still undefined: consider step 3; consider protocol 3; repeat total IgG, IgA, IgM and IgG-subclasses after 1–2 years, and booster responses after 3–5 years. Abnormal CH50 and/or AP50: determination of individual complement components (e.g. C1q, C2, C4, C5–C9, properdin, factor B/I/H). ANA. In case of angioedema: C1-inhibitor function (if level is normal). Genetic determination of defect if possible.</td>
</tr>
<tr>
<td><strong>Abnormal results step 3</strong></td>
<td>Follow appropriate work-up guided by clinical presentation and laboratory results. Genetic determination of defect if possible.</td>
</tr>
</tbody>
</table>

Protocol 1. ANA: anti-nuclear antibody; C: complement; CD: cluster of differentiation; Ig: immunoglobulin; MBL: mannose binding lectin; PID: primary immunodeficiency. Orange shading: consultation with an immunologist is highly recommended.
### Protocol 2

#### Step 1
**Don’t hesitate to rule out SCID and AIDS**

**Perform**
- Blood count and differential (check platelet volume, absolute lymphocyte, neutrophil and eosinophil counts; IgG, IgA, and IgM; IgE; lymphocyte subpopulations (Table 4); tests for HIV.

**Next step**
- **HIV-positive**: treat accordingly. *Agammaglobulinaemia, lymphocytopenia: go to step 2a. Normal results, but no improvement, no other diagnosis: go to step 2a. The possibility of SCID is an emergency! Early SCT can save lives.*

#### Step 2a
**Combined T and B cell immunodeficiencies**

**Perform**
- Lymphocyte subpopulations and proliferation tests (Table 4). Consider lymphocyte subpopulations using a more extended protocol than the one mentioned in Table 4. *Hypogammaglobulinaemia: consider secondary causes; add IgG-subclasses, booster responses, M-proteins.*

**Next step**
- **Abnormal results**: go to step 4. **Normal results**: consider step 3, consider protocol 3.

#### Step 2b
**Identify T lymphocyte-macrophage communication defects**

**Perform**
- T lymphocyte/macrophage communication (IL-12, IL-12-receptor, IFN-γ-receptor, STAT1) by referral to specialist centre.

**Next step**
- **Normal results**: go to step 1, if not yet performed. Consider step 3. Consider protocol 3. **Abnormal results**: genetic determination of defect if possible.

#### Step 3
**Other potential PIDs**

**Normal results steps 1 & 2**
- *When symptoms or signs from Table 1 are present: consult an immunologist to determine a specific work-up. Other potential explanations for recurrent infections do not always automatically exclude PID.*

#### Step 4
**Final diagnosis**

**Clinical status**
- Test for chimerism (maternal T-lymphocytes). Analyse and treat possible infections (consider viral PCR/culture/serology, BAL, organ biopsy for histology and culture; look for opportunistic pathogens with appropriate techniques); serology is unreliable!

**Immune system**
- Consider *in vitro* cytokine production, *in vivo* functional tests (e.g. stimulation with neoantigen; PPD or candida skin tests), analysis of bone marrow, lymph node biopsy. NK cell cytotoxicity.

**Underlying defect**
- Consider uric acid, ADA, PNP, α-fetoprotein, X-ray of long bones if short stature or disproportional growth, thymus size (chest X-ray, ultrasound), chromosomal analysis, radiosensitivity tests, 22q11 analysis, clonality studies (Vβ-gene usage). Determination of genetic defect if possible.

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*Protocol 2. ADA: adenosine deaminase; AIDS: acquired immunodeficiency syndrome; BAL: bronchoalveolar lavage; CD: cluster of differentiation; HIV: human immunodeficiency virus; Ig: immunoglobulin; IFN: interferon; IL: interleukin; NK: natural killer; PID: primary immunodeficiency; PNP: purine nucleoside phosphorylase; PPD: purified protein derivative; SCID: severe combined immunodeficiency; SCT: stem cell transplantation; STAT: signal transducers and activators of transcription. Orangeshading: consultation with an immunologist is highly recommended.*
Table 3. Age-related reference values for lymphocyte subpopulations (p10 – p90)*

<table>
<thead>
<tr>
<th>Lymphocyte subpopulation</th>
<th>neonate</th>
<th>1 w - 2 m</th>
<th>2 - 5 m</th>
<th>5 - 9 m</th>
<th>9 - 15 m</th>
<th>15 - 24 m</th>
<th>2 - 5 y</th>
<th>5 - 10 y</th>
<th>10 - 16 y</th>
<th>adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>2.2 - 6.9</td>
<td>4.0 - 11.3</td>
<td>3.9 - 8.6</td>
<td>4.0 - 9.0</td>
<td>3.1 - 8.7</td>
<td>3.4 - 8.9</td>
<td>2.3 - 5.6</td>
<td>1.6 - 4.3</td>
<td>1.3 - 3.0</td>
<td>1.1 - 2.5</td>
</tr>
<tr>
<td>CD19+ B-lymphocytes</td>
<td>0.2 - 1.0</td>
<td>0.3 - 1.7</td>
<td>0.8 - 2.6</td>
<td>0.8 - 2.2</td>
<td>0.7 - 2.4</td>
<td>0.9 - 2.5</td>
<td>0.4 - 1.5</td>
<td>0.3 - 0.7</td>
<td>0.2 - 0.5</td>
<td>0.1 - 0.4</td>
</tr>
<tr>
<td>CD3+ T-lymphocytes</td>
<td>1.1 - 4.2</td>
<td>2.8 - 6.5</td>
<td>2.4 - 5.6</td>
<td>2.7 - 6.1</td>
<td>1.8 - 5.9</td>
<td>2.2 - 5.5</td>
<td>1.4 - 3.6</td>
<td>1.1 - 2.8</td>
<td>1.0 - 2.0</td>
<td>0.7 - 1.9</td>
</tr>
<tr>
<td>CD3+ /CD4+ helper T-lymphocytes</td>
<td>0.8 - 2.9</td>
<td>2.1 - 4.9</td>
<td>1.6 - 4.2</td>
<td>1.7 - 4.1</td>
<td>1.3 - 4.1</td>
<td>1.1 - 3.6</td>
<td>0.7 - 2.0</td>
<td>0.5 - 1.8</td>
<td>0.5 - 1.3</td>
<td>0.4 - 1.3</td>
</tr>
<tr>
<td>CD3+/CD8+ cytotoxic T-lymphocytes</td>
<td>0.3 - 1.8</td>
<td>0.5 - 1.6</td>
<td>0.7 - 1.5</td>
<td>0.7 - 1.8</td>
<td>0.5 - 1.6</td>
<td>0.5 - 1.8</td>
<td>0.5 - 1.4</td>
<td>0.4 - 1.2</td>
<td>0.3 - 0.8</td>
<td>0.2 - 0.7</td>
</tr>
<tr>
<td>CD3+/TCR + T-lymphocytes</td>
<td>0.03 - 0.2</td>
<td>0.02 - 0.2</td>
<td>0.08 - 0.3</td>
<td>0.09 - 0.3</td>
<td>0.07 - 0.4</td>
<td>0.08 - 0.5</td>
<td>0.1 - 0.3</td>
<td>0.07 - 0.5</td>
<td>0.04 - 0.2</td>
<td>0.02 - 0.2</td>
</tr>
<tr>
<td>CD3-/CD16,56+ NK-cells</td>
<td>0.2 - 1.8</td>
<td>0.3 - 0.8</td>
<td>0.2 - 0.9</td>
<td>0.2 - 0.8</td>
<td>0.2 - 0.8</td>
<td>0.2 - 0.9</td>
<td>0.1 - 1.1</td>
<td>0.1 - 0.7</td>
<td>0.1 - 0.6</td>
<td>0.1 - 0.7</td>
</tr>
</tbody>
</table>

* Comans-Bitter WM et al., Rotterdam, 2006. Adapted from: Comans-Bitter WM et al., Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr 1997;130:388-93. w = week, m = months, y = years.
Table 4. Basic protocol for in vitro determination of lymphocyte subpopulations and function

a) Determine the absolute count of the following lymphocyte subpopulations, and compare the results with age-matched reference values

<table>
<thead>
<tr>
<th>CD Antigen</th>
<th>Subpopulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>T-lymphocytes</td>
</tr>
<tr>
<td>CD3/CD4+</td>
<td>Helper-T-lymphocytes</td>
</tr>
<tr>
<td>CD3/CD4+/CD27+/CD45RA+</td>
<td>Naive helper-T lymphocytes</td>
</tr>
<tr>
<td>CD3/CD8+</td>
<td>Cytotoxic T-lymphocytes</td>
</tr>
<tr>
<td>CD3/HLA-DR+</td>
<td>Activated T-lymphocytes</td>
</tr>
<tr>
<td>CD3/TCR-α/CD4/CD8+</td>
<td>‘Double-negative’ T-lymphocytes</td>
</tr>
<tr>
<td>CD3/TCR-β+</td>
<td>TCR-β+ subset of T-lymphocytes</td>
</tr>
<tr>
<td>CD19+ or CD20+ B</td>
<td>B-lymphocytes</td>
</tr>
<tr>
<td>CD19+/CD27+IgM+IgD-</td>
<td>Switched memory-B lymphocytes</td>
</tr>
<tr>
<td>CD3/CD16+ and/or CD56+</td>
<td>NK-cells</td>
</tr>
</tbody>
</table>

b) Determine the uptake of [3H]-thymidine (or CFSE or activation markers) and compare the results with, preferably, age-matched controls after stimulation with:

- Mitogens (e.g. PHA, PMA + ionomycin, PWM).
- Consider monoclonal antibodies (e.g. CD2 ± CD28, CD3 ± CD28).
- Antigens (e.g. tetanus, after booster vaccination; PPD, candida).
- Consider allogeneic cells.

Part (a) can be performed in many hospitals, part (b) is performed in specialized laboratories only. For correct interpretation of the results, collaboration with an immunologist specialized in immunodeficiency and/or a specialized laboratory is highly recommended.

CD: cluster of differentiation; CFSE: carboxyfluorescein succinimidyl ester; HLA: human leucocyte antigen; NK: natural killer; PHA: phytohaemagglutinin; PMA: phorbolmyristate acetate; PWM: pokeweed mitogen; TCR: T cell receptor.

Table 5. Protocol for determination of granulocyte function

a) Oxidative burst and flow cytometry

Flow cytometric analysis using dihydrorhodamine (DHR)
Nitroblue tetrazolium test (NBT) to a stimulant (PMA, LPS)
Chemoluminescence test
Immunophenotyping (CD18, CD11b, sLeX, kindlin3)

b) Chemotaxis, granule contents, bacterial killing, phagocytosis

Migration to a chemoattractant (e.g. FMLP)
Immunohistochemistry of granule contents, electron microscopy
Bacterial killing (e.g. of Staphylococcus aureus)
Phagocytosis (e.g. of zymosan uptake, FITC-conjugated latex beads)

Part (a) can be performed in many hospitals, part (b) is performed in specialized laboratories only. For correct interpretation of the results, collaboration with an immunologist specialized in immunodeficiency and/or a specialized laboratory is highly recommended.


Table 6. Age-matched reference values of serum immunoglobulins and IgG subclasses (in g/l)**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 2 weeks</td>
<td>6.5 - 12.6</td>
<td>&lt;0.16</td>
<td>0.03 - 0.24</td>
</tr>
<tr>
<td>0.5 - 4 months</td>
<td>2.6 - 7.8</td>
<td>0.06 - 0.57</td>
<td>0.10 - 0.55</td>
</tr>
<tr>
<td>4 - 6 months</td>
<td>2.2 - 11.3</td>
<td>0.08 - 0.90</td>
<td>0.07 - 0.65</td>
</tr>
<tr>
<td>6 - 24 months</td>
<td>2.6 - 15.2</td>
<td>0.16 - 1.1</td>
<td>0.10 - 1.2</td>
</tr>
<tr>
<td>2 - 6 year</td>
<td>4.3 - 13.4</td>
<td>0.19 - 2.2</td>
<td>0.21 - 1.8</td>
</tr>
<tr>
<td>6 - 16 year</td>
<td>5.2 - 15.6</td>
<td>0.54 - 3.6</td>
<td>0.13 - 2.4</td>
</tr>
<tr>
<td>Adults</td>
<td>7.0 - 16.0</td>
<td>0.70 - 4.0</td>
<td>0.40 - 2.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age Group</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1 months</td>
<td>2.4 - 10.6</td>
<td>0.87 - 4.1</td>
<td>0.14 - 0.55</td>
<td>0.039 - 0.56</td>
</tr>
<tr>
<td>1 - 4 months</td>
<td>1.8 - 6.7</td>
<td>0.38 - 2.1</td>
<td>0.14 - 0.70</td>
<td>0.022 - 0.36</td>
</tr>
<tr>
<td>4 - 6 months</td>
<td>1.8 - 7.0</td>
<td>0.34 - 2.1</td>
<td>0.15 - 0.80</td>
<td>0.017 - 0.23</td>
</tr>
<tr>
<td>6 - 24 months</td>
<td>2.0 - 8.5</td>
<td>0.34 - 2.6</td>
<td>0.15 - 1.13</td>
<td>0.011 - 0.79</td>
</tr>
<tr>
<td>2 - 6 year</td>
<td>3.2 - 10.0</td>
<td>0.52 - 3.4</td>
<td>0.13 - 1.33</td>
<td>0.012 - 1.58</td>
</tr>
<tr>
<td>6 - 18 year</td>
<td>3.7 - 12.8</td>
<td>0.85 - 6.1</td>
<td>0.13 - 1.63</td>
<td>0.023 - 2.3</td>
</tr>
<tr>
<td>Adults</td>
<td>4.9 - 11.4</td>
<td>1.50 - 6.4</td>
<td>0.20 - 1.10</td>
<td>0.080 - 1.40</td>
</tr>
</tbody>
</table>


Since 2006, many new PIDs have been identified; the IUIS Expert Committee on PIDs published updates of their classification. We have therefore updated the multi-stage diagnostic protocol which was started in 2006 by the Clinical Working Party of ESID. Because evidence supporting diagnostic decisions is still limited, the protocols are based largely on consensus of expert opinions. The protocol starts from the clinical presentation of both paediatric and adult patients. The multi-stage design allows timely identification of potential PID by all doctors, while more costly elaborate tests are reserved for definitive classification at a later stage, in collaboration with an immunologist specialized in the field of immunodeficiency and a specialized laboratory.

Do not forget PID and pick up the signs: it is life-saving
Considering the possibility of a PID is the key to the diagnosis. The incidence of PIDs differs depending on the disease; all PIDs taken together may be as frequent as 1:2000. The diagnostic process starts with symptoms from the history (Table 1A), signs on physical examination (Table 1B) and baseline blood tests (Table 1C). Successful treatment with prevention of organ damage is dependent upon rapid recognition. Family history is a vital clue to the diagnosis of PID; this also holds true for adult patients who can present with late-onset forms of disease.

Pattern recognition is the key to identification
PIDs tend to present in one of eight different clinical presentations (Table 2, column 1), determined by the underlying pathology of the disease. Either initially or during follow-up some patients may show features of more than one clinical presentation, which can be confusing. Encountered pathogens (Table 2, column 2) can help to clarify the pattern, because specific immunological defects will lead to particular patterns of infection. Associated features (Table 2, column 3) and age of presentation can also help. In column 5 of Table 2, directions towards the appropriate multi-stage diagnostic protocol for suspected immunodeficiency (Protocols 1–3) are given, using the clinical presentation as the starting-point. In the protocols, severe defects are ruled out first with widely available screening tests (step 1). Less severe forms of PID can be diagnosed later (steps 2–4), after more frequent non-immunological diseases have been ruled out (Table 2, column 4). It is essential to use age-matched reference values (Tables 3 and 6) to avoid misinterpreting test results, especially in young infants who normally have a relative lymphocytosis and a high level of maternal immunoglobulins in their blood. Secondary immunodeficiencies present in a similar fashion to PIDs. Also, drugs, malignancies and diseases which cause protein and/or lymphocyte loss may cause secondary immunodeficiency. It is important to eliminate these possibilities before making a definitive diagnosis of PID. Many new PIDs have been identified in the past decades, and more are likely in the near future, so this multi-stage diagnostic protocol will need to be revised from time to time.

Protocols
Most patients with PID have recurrent ENT and airway infections, but most patients with recurrent ENT and airway infections do not have PID. If a PID is present, it usually concerns a defect in specific antibody production or complement. Both types of defects result in decreased opsonization of bacteria and consequently decreased phagocytosis. These defects are analyzed in Protocol 1. Patients with unusual or opportunistic infections and/or failure to thrive may suffer from a severe life-threatening immunodeficiency. Generally, this concerns a defect related to T-lymphocytes. These defects are analyzed in Protocol 2. In Protocol 3, patients with recurrent pyogenic infections are analyzed. In this group of patients a phagocyte defect can be expected, which is usually caused by a decreased phagocyte count related to autoimmune disease or iatrogenic causes. Only seldom a functional phagocyte disorder will be found.

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Visit www.PIDinteractive.eu, the interactive web application based on the tables and protocols from the publication which will guide you through the various steps.

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Take-home messages
- The key to detect a PID is to consider the possibility.
- PIDs almost always present with one or more of eight clinical presentations; these can be used as the starting-point to enter the appropriate diagnostic protocol.
- SCID is an emergency.
- Timely recognition of antibody deficiency prevents future organ damage.
- If PID is suspected or runs in the family, delay live-attenuated vaccinations and do not postpone immunological investigations.
- Use age-matched reference values to avoid misinterpretation of immunological test results.

www.PIDinteractive.eu