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

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Summary

Members of the European Society for Immunodeficiencies (ESID) and other colleagues have updated the multi-stage expert-opinion-based diagnostic protocol for non-immunologists incorporating newly defined primary immunodeficiency diseases (PIDs). The protocol presented here aims to increase the awareness of PIDs among doctors working in different fields. Prompt identification of PID is important for prognosis, but this may not be an easy task. The protocol therefore starts from the clinical presentation of the patient. Because PIDs may present at all ages, this protocol is aimed at both adult and paediatric physicians. The multi-stage design allows cost-effective screening for PID of the large number of potential cases in the early phases, with more expensive tests reserved for definitive classification in collaboration with a specialist in the field of immunodeficiency at a later stage.

Keywords: diagnostic protocol, immunological evaluation, primary immunodeficiency, update

Introduction

In 2006, the Clinical Working Party of the European Society for Immunodeficiencies (ESID) published a multi-stage diagnostic protocol suitable for all doctors [1]. The protocol started from the clinical presentation of both paediatric and adult patients. Many primary immunodeficiency diseases (PIDs) present in childhood, but the most common clinically significant PID, 'common variable immunodeficiency disorders' (CVID), has a peak onset in the second and third decades of life. The multi-stage design allowed timely identification of potential PID by all doctors, while more costly elaborate tests were reserved for definitive classification at a later stage, in collaboration with an immunologist specialized in the field of immunodeficiency and a specialized laboratory.

Since 2006, many new PIDs have been identified; the International Union of Immunological Societies (IUIS) Expert Committee on Primary Immunodeficiencies published updates of their classification of PIDs, the latest in 2009 [2]. We have therefore updated the 2006 diagnostic protocol, using the IUIS 2009 paper and its references as the basis for clinical disease entities of PIDs. Additionally, a PubMed search was performed from 2007 onwards; several papers discussing the recognition of potential PID in everyday practice were found [3–13], and all were based mainly on expert opinion. All ESID members received an invitation to participate in this effort. [Searchstrategy, papers selected for algorithms designed for identification of potential PID patients in everyday clinical practice published in English in international papers: 1. 'Related citations' for the original paper [1] (three relevant hits, references [3–5]); 'Immunologic Deficiency Syndromes/*classification[MeSH] NOT HIV NOT AIDS NOT HTLV NOT Simian' (no additional relevant hits); 'Immunologic Deficiency Syndromes/*diagnosis[MeSH] NOT HIV NOT AIDS NOT HTLV NOT Simian' (eight additional relevant hits, including the original ESID paper, references [1,4,6–11]); two additional papers suggested by contributors (references [12,13]).]

While the general outline of the diagnostic protocol has remained the same, novel PIDs have been incorporated. The body of knowledge concerning PIDs has expanded considerably; therefore, possible diagnoses are now presented separately from the clinical protocols. Because evidence supporting diagnostic decisions is still limited, the protocols are based largely on consensus of expert opinions.

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. Unfortunately, the awareness of PIDs among

professionals is low, as PIDs are considered rare and complex diseases. However, the incidence of PIDs ranges – depending on the disease – from 1:500 for often asymptomatic immunoglobulin (Ig)A deficiency to 1:500 000 [14,15]; all PIDs taken together may be as frequent as 1:2000 [16]. Like any other diagnostic process, symptoms from the history (Table 1a), signs on physical examination (Table 1b) and baseline blood tests (Table 1c) should alert any physician to the possibility of PID in children and adults, even though they are unfamiliar with the precise possible diagnosis.

This is important, as successful treatment of a child with severe PID such as severe combined immunodeficiency (SCID) is dependent upon rapid recognition [17]. Non-immunologists such as general paediatricians play a vital role. Leucocyte differential and immunoglobulin isotype levels enable detection in most cases; these can be performed in many hospitals. Less urgent, but still important if future organ damage and decreased quality of life and lifespan are to be prevented, is the timely recognition of late-onset as well as less pronounced forms of PID in older children and adults [18]. Non-immunologists such as primary care physicians, general paediatricians, pulmonologists and ear, nose and throat (ENT) specialists play an important role here. Common examples are antibody deficiencies such as CVID and specific anti-polysaccharide antibody deficiency (SPAD) [19,20]. These generally present with recurrent respiratory infections, by far the most common clinical presentation of PID. Confusingly, this clinical presentation is often encountered in everyday practice, especially in young children, but also in older children and adults in any pulmonology or ENT service. Most of these patients do not have PID. However, when more than one pneumonia occurs, bronchiectasis is present, the infections fail to clear with conventional treatment or continue to occur when a young child grows older, immunological investigations are needed, and consultation of an immunologist is highly recommended.

Family history is a vital clue to the diagnosis of PID, as although patients with recurrent infections do not often have PID, this becomes much more likely when it 'runs in the family'. 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 (Table 3). 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 [21]. Associated features (Table 2, column 3) and age of presentation can also help. Most PIDs present in childhood but due to, for example, hypomorphic mutation, typical paediatric disease may present later [22]. CVID is the most common PID presenting in adulthood [5].

In column 5 of Table 2, directions towards the appropriate multi-stage diagnostic protocol for suspected immunodeficiency (Figs 1–3; Tables 4 and 5) 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; Figs 1–3). Less severe forms of PID can be diagnosed later (steps 2–4; Figs 1–3), after more frequent non-immunological diseases have been ruled out (Table 2, column 4). It is essential to use age-matched reference values [23–25] 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. Beyond the first step of each protocol, and in all cases where a severe PID such as SCID is suspected, timely collaboration with an immunologist to decide on further diagnostic steps and to aid with the interpretation of the results is highly recommended.

Secondary immunodeficiencies present in a similar fashion to PIDs. Human immunodeficiency virus (HIV) infection occurs much more frequently in some parts of the world. Also, drugs, malignancies and diseases which cause protein and/or lymphocyte loss may cause secondary immunodeficiency; this is more common than unrecognized PID in adults [5]. 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.

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.

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Table 1. Symptoms and signs that could point to potential PID.

(a) History

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)

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

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
Hypersensitivity to sunlight
Hypocalcaemic 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.

	Clinical presentation	Encountered pathogens	Special features	Non-immunological differential diagnosis	Diagnostic protocol
1	Recurrent ENT and airway infections (including bronchiectasis) 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 >1 pneumonia occurs, or when ENT infections persist abnormally long	Mainly extracellular bacteria such as *Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catharralis* Sometimes: *Staphylococcus aureus, Neisseria meningitidis*, group A *Streptococcus, Mycoplasma pneumoniae, Ureaplasma urealyticum, Campylobacter jejuni, Helicobacter pylori* Diarrhoea due to *Giardia lamblia.*	Bronchiectasis. Recurrent bronchitis in a non-smoker. Unexplained chronic cough. Chronic sinusitis (Enteroviral meningoencephalitis is a severe complication in inadequately substituted agammaglobulinaemia)	Frequent, children: normal frequency of infection in infants (day-care, passive smoking), bronchial hyperreactivity, allergy, asthma, adenoidal hypertrophy, iron deficiency anaemia, gastro-oesophageal reflux Frequent, adults: COPD Infrequent, children: cystic fibrosis, inhaled foreign body, congenital anomaly, BPD; intestinal or renal protein loss Infrequent, adults: cystic fibrosis; intestinal or renal protein loss. Rare, children and adults: ciliary dyskinesia, α1-anti-trypsin deficiency	Go to protocol 1
2	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.	Mainly viruses (CMV, EBV, VZV, HSV, adenovirus, HHV8, HPV, molluscum contagiosum, RSV), fungi (superficial *Candida, Aspergillus, Cryptococcus, Histoplasma, Pneumocystis jiroveci/carinii*), protozoa (*Toxoplasma, Microsporidium, Cryptosporidium*) and intracellular bacteria such as *Mycobacterium* spp. and *Salmonella*	Intractable diarrhoea with or without identified pathogen Unusual infections or unusually severe course of infections, opportunistic infections Graft-*versus*-host reaction from maternal T lymphocytes or non-irradiated blood transfusion Severe eczema Photosensitivity	A variety of gastrointestinal, renal, cardiopulmonary, endocrine, neurological, metabolic and congenital causes. Malignancy. Chronic lead poisoning. Perinatal infection. Severe malnutrition (see appropriate textbooks)	Go to protocol 2
3	Recurrent pyogenic infections (including granulomatous inflammation, poor wound healing) Defects in phagocyte function are rare and seldom immediately life-threatening. Neutropenia is more common and easily detected	Mainly *Staphylococcus aureus*, sometimes *Klebsiella, Escherichia coli, Enterobacter, Serratia, Pseudomonas, Salmonella, Chromobacterium violaceum, Burkholderia* species. Invasive fungal infection (disseminated *Candida, Aspergillus, Nocardia*)	Infections of body surface areas (skin, mouth, mucous membranes), abscesses of internal organs (lung, liver, lymph nodes, gut) and bones. Unexplained granulomatous inflammation. Poor wound healing. Aphthae. Granulomatous colitis with severe rectal involvement. Delayed separation of umbilical cord (>4 weeks)	Neutropenia due to drugs; alloimmune, autoimmune; haematological malignancy, aplastic anaemia. Transient neutropenia following (viral) infections. Vitamin B12/folate deficiency Disrupted skin (eczema, burns)	Go to protocol 3
4	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	Mainly intracellular bacteria such as *Mycobacterium* spp. and *Salmonella*, viruses (CMV, EBV, VZV, HSV, JC, HPV), fungi (Candida, Aspergillus, Cryptococcus, Histoplasma, *Pneumocystis jiroveci/carinii*) and protozoa (Toxoplasma, Microsporidium, Cryptosporidium).	May present later in life Early onset; association of multiple features; atypical resistance to treatments; opportunistic infections	Virulent strain of pathogen, reduced general condition of patient leading to secondary immunodeficiency (malignancy, malnutrition, chronic disease). Immunosuppressive therapy. HIV	Go to protocol 2
5	Recurrent infections with the same type of pathogen Many have no PID, but the recurrent infections may be life-threatening. Screening is therefore warranted	Intracellular bacteria such as *Salmonellae, mycobacteria* *Neisseriae* such as *meningococci* Yeasts, fungi such as *candida* Encapsulated bacteria such as *pneumococci* Viruses	Normally no other recurrent infectious problems No/delayed fever/raise in CRP: deficiency in NF-κB signalling (IRAK4, NEMO-ID, IκBα deficiency). Encapsulated bacterial sepsis: asplenia Excessive warts: epidermodysplasia verruciformis, WHIM, DOCK8 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	Intracellular bacteria: go to protocol 2 step 2b (T lymphocyte–macrophage interaction for cytokine production; autoantibodies to IFN-γ) *Neisseriae*: go to protocol 1 (complement deficiency, sometimes antibody deficiency) Yeasts, fungi: go to protocol 2 (T lymphocyte deficiency, CMC, MPO) Encapsulated bacteria: go to protocol 1 (antibody deficiency, IRAK4 deficiency, complement deficiencies) Viruses: go to protocol 2

Table 2. *Continued*

	Clinical presentation	Encountered pathogens	Special features	Non-immunological differential diagnosis	Diagnostic protocol
6	Autoimmune or chronic inflammatory disease; lymphoproliferation Most cases of autoimmune disease, 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 conditions including autoimmune diseases, acute phase reactants, lymphoproliferation. Identify by clinical features. Atypical HUS. Unexplained haemolysis	(See appropriate textbooks.)	Start with protocol 1, 2 or 3 guided by predominant clinical presentation (1–5, see above) When in doubt perform a combination of the tests in steps 1 from all three protocols
7	Characteristic combinations of clinical features (eponymous syndromes) 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. See ref [26].)	Follow appropriate protocol guided by predominant clinical presentation (1–6, 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
8	Angioedema	–	Related to triggering factors (e.g. stress, trauma, menses) Symptoms typically last >24 h. Not reacting to epinephrine/antihistamine/ corticosteroid treatment. May mimic acute abdomen	Allergy, malignancy, auto-immunity ACE-inhibitor therapy Idiopathic	Go to protocol 1 step 2b

Columns 1 and 5 are the core of the table, and can be used to go directly to the appropriate diagnostic protocol, guided solely by the clinical presentation of the patient. Columns 2 and 3 contain extra information that can be useful, but does not necessarily have to be used. Column 4 contains information on the non-immunological differential diagnosis. ACE, angiotensin-converting enzyme; BPD, bronchopulmonary dysplasia; CMC, chronic mucocutaneous candidiasis; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disorder; CRP, C-reactive protein; EBV, Epstein-Barr virus; ENT, ear-nose-throat; HHV8, human herpes virus 8; HIV, human immunodeficiency virus; HPV, human papilloma virus; HSV, herpes simplex virus; HUS, haemolytic uraemic syndrome; IRAK4, interleukin-1 receptor-associated kinase 4; JC, JC virus; MPO, myeloperoxidase; NEMO-ID, X-linked mutations in nuclear factor (NF)-κB essential modulator with immune deficiency and often ectodermal dysplasia with anhydrosis (EDA); NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK, natural killer; PID, primary immunodeficiency disease; RSV, respiratory syncytial virus; SCT, stem cell transplantation; VZV, varicella zoster virus, WHIM, warts, hypogammaglobulinemia, infections and myelokathexis syndrome.

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Table 3. In-depth differential diagnosis of the clinical presentations.

Clinical presentations		Suspected category of immunodeficiency [3] (same order as IUIS tables; bold: most frequent)	Possible immunological diagnosis [3] (same order and designation as IUIS tables; bold: most frequent)
1	Recurrent ENT and airway infections (unexplained bronchiectasis)	Combined T and B cell immunodeficiencies	DOCK8
		Predominantly antibody deficiencies	Severe reduction in all serum immunoglobulin isotypes with profoundly decreased or absent B cells (Btk, μ heavy chain, λ5, Igα, Igβ, BLNK, thymoma with immunodeficiency) Severe reduction in at least two serum immunoglobulin isotypes with normal or low numbers of B cells (**CVIDs**, ICOS, CD19, **TACI**, BAFF-R) Severe reduction in serum IgG and IgA with normal/elevated IgM and normal numbers of B cells (CD40L, CD40, AID, UNG) Isotype or light chain deficiencies with normal numbers of B cells (**Ig heavy chain**, κ chain, **isolated IgG subclass**, **IgA with IgG subclass**, **selective IgA**) **Specific antibody deficiency with normal Ig concentrations and normal numbers of B cells** **Transient hypogammaglobulinaemia of infancy with normal numbers of B cells**
		Other well-defined immunodeficiency syndromes	PMS2; AR-HIES
		Congenital defects of phagocyte number, function, or both	P14; pulmonary alveolar proteinosis
		Defects in innate immunity	NEMO-ID; IRAK4; MyD88; warts, hypogammaglobulinaemia, infections, myelokathexis syndrome (WHIM)
		Complement deficiencies	Complement deficiency (C1q, C1r, C4, C2, C3, factor I, MBP, MASP2); immunodeficiency associated with ficolin 3 deficiency.
2	Failure to thrive from early infancy (intractable diarrhoea, severe eczema)	**Combined T and B cell immunodeficiencies**	T–B + SCID (γc, JAK3, IL7-Rα, CD45, CD3δ, CD3ε, CD3ζ, Coronin-1a); T–B – SCID (RAG1/2, DCLRE1C (Artemis), DNA PKcs, ADA, reticular dysgenesis); Omenn syndrome; DNA-ligase IV; Cernunnos; PNP; CD3γ; CD8; ZAP-70; Ca^{++} channel; MHC class I; MHC class II; winged helix (nude), FOXN1; CD25; STAT5b
		Other well-defined immunodeficiency syndromes	Thymic defects (**DiGeorge**, **22q11.2 deletion**, 10p deletion); immune-osseous dysplasias (cartilage hair hypoplasia, Schimke); Comel–Netherton
		Congenital defects of phagocyte number, function, or both	IFN-γ-receptor-1 (mainly recessive complete disorder).
		Diseases of immune dysregulation	IPEX
		Defects in innate immunity	NEMO-ID
3	Recurrent pyogenic infections (granulomatous inflammation, poor wound healing)	Other well-defined immunodeficiency syndromes	AD-HIES (Job syndrome) (STAT3)
		Congenital defects of phagocyte number, function, or both	Severe congenital neutropenias (ELA2, GFI1); Kostmann; neutropenia with cardiac and urogenital malformations (G6PC3); glycogen storage disease type 1b; cyclic neutropenia; X-linked neutropenia/myelodysplasia; P14; LAD1; LAD2; LAD3; rac2; β-actin; localized juvenile periodontitis; Papillon–Lefèvre syndrome; specific granule deficiency; Shwachman–Diamond syndrome; **CGD** (**X-linked**, CYBB; **autosomal**, CYBA, NCF1/2) G6PD, MPO
		Defects in innate immunity	NEMO-ID; warts, hypogammaglobulinaemia, infections, myelokathexis syndrome (WHIM)
		Complement deficiencies	Complement deficiency (C3, Factor I); immunodeficiency associated with ficolin 3 deficiency
4	Unusual infections or unusually severe course of infections (unexplained – periodic fever see 6)	Combined T and B cell immunodeficiencies	T-B + SCID (γc, JAK3, IL7-Rα, CD45, CD3δ, CD3ε, CD3ζ, Coronin-1A); T-B- SCID (RAG1/2, DCLRE1C (Artemis), DNA PKcs, ADA, reticular dysgenesis); Omenn syndrome; DNA-ligase IV; Cernunnos; CD40 ligand; CD40; PNP; CD3γ; CD8; ZAP-70; Ca ++ channel; MHC class I; MHC class II; winged helix (nude), FOXN1; CD25; STAT5b; ITK; DOCK8.
		Other well-defined immunodeficiency syndromes	Wiskott-Aldrich syndrome; immunodeficiency with centromeric instability and facial anomalies (ICF); thymic defects (**DiGeorge**, **22q11.2 deletion**, 10p deletion); immune-osseous dysplasias (cartilage hair hypoplasia, Schimke); Comel-Netherton; HIES; hepatic venoocclusive disease with immunodeficiency (VODI); XL-dyskeratosis congenita (Hoyeraal-Hreidarsson syndrome).
		Diseases of immune dysregulation	FHL; XLP.
		Defects in innate immunity	NEMO-ID; warts, hypogammaglobulinaemia, infections, myelokathexis syndrome (WHIM).

Table 3. *Continued*

Clinical presentations		Suspected category of immunodeficiency [3] (same order as IUIS tables; bold: most frequent)	Possible immunological diagnosis [3] (same order and designation as IUIS tables; bold: most frequent)
5	Recurrent infections with the same type of pathogen	Other well-defined immunodeficiency syndromes	AD-HIES (Job syndrome); AR-HIES; chronic mucocutaneous candidiasis.
		Congenital defects of phagocyte number, function, or both	IL-12 and IL-23 receptor β1 chain; IL-12p40; IFN-γ-receptor1/2; AD hyper-IgE; hyper-IgE (STAT3, TYK2); MPO
		Defects in innate immunity	Epidermodysplasia verruciformis; herpes simplex encephalitis (HSE); trypanosomiasis (APOL-1)
		Complement deficiencies	Complement deficiency (C2, C3, C5, C6, C7, C8, C9, properdin)
6	Autoimmune or chronic inflammatory disease; lymphoproliferation	Combined T and B cell immunodeficiencies	Omenn syndrome; CD25; STAT5b; ITK
		Predominantly antibody deficiencies	**CVIDs**
		Other well-defined immunodeficiency syndromes	Wiskott–Aldrich syndrome; Nijmegen breakage syndrome; PMS2
		Diseases of immune dysregulation	Immunodeficiency with hypopigmentation (Chediak–Higashi syndrome, Griscelli syndrome type 2, Hermanski–Pudlak syndrome type 2); familial haemophagocytic lymphohistiocytosis (FHL) syndromes (Perforin, UNC13D, Syntaxin11, STXBP2); lymphoproliferative syndromes (XLP1 (SH2D1A), XLP2 (XIAP), ITK); syndromes with autoimmunity, autoimmune lymphoproliferative syndrome (ALPS) (CD95, CD95L, caspase 8, caspase 10, activating N-ras defect); APECED; IPEX.
		Congenital defects of phagocyte number, function, or both	X-linked neutropenia/myelodysplasia; pulmonary alveolar proteinosis
		Autoinflammatory disorders	**FMF**; TRAPS, hyper-IgD syndrome; Muckle–Wells syndrome; familial cold autoinflammatory syndrome; neonatal onset multi-system inflammatory disease (NOMID)/chronic infantile neurologic cutaneous and articular syndrome (CINCA); pyogenic sterile arthritis, pyoderma gangrenosum, acne (PAPA) syndrome; Blau syndrome; chronic recurrent multi-focal osteomyelitis and congenital dyserythropoietic anaemia (Majeed syndrome); deficiency of the IL-1 receptor antagonist (DIRA)
		Complement deficiencies	Complement component deficiency (**C1q**, C1r, C1s, **C4**, C2, C3, C5, C6, C7, C8, C9; **PNH** (CD55/CD59 deficiency)
7	Characteristic combinations of clinical features (eponymous syndromes)	Combined T and B cell immunodeficiencies	Omenn syndrome; DNA-ligase IV; Cernunnos; PNP; winged helix (nude), FOXN1
		Other well-defined immunodeficiency syndromes	**Ataxia telangiectasia**; ataxia telangiectasia-like disease (ATLD); Nijmegen breakage syndrome; Bloom syndrome; immunodeficiency with centromeric instability and facial anomalies (ICF); **Di-George syndrome**; immune-osseous dysplasias (cartilage hair hypoplasia, Schimke, Comel–Netherton); XL-dyskeratosis congenita (Hoyeraal–Hreidarsson syndrome)
		Diseases of immune dysregulation	Immunodeficiency with hypopigmentation (Chediak–Higashi syndrome, Griscelli syndrome type 2, Hermanski–Pudlak syndrome type 2).
		Congenital defects of phagocyte number, function, or both	P14; LAD2; β-actin; Shwachman–Diamond
		Defects in innate immunity	NEMO-ID
		Autoinflammatory disorders	NOMID / CINCA, Blau, Majeed syndromes
8	Angioedema	Complement deficiencies	**C1-inhibitordeficiency**.

This table contains additional information for those interested; this information is not needed for the initial diagnostic evaluation process. For explanations concerning the various immunological disorders the reader is referred to the original IUIS 2009 publication [2] and its references; the word 'deficiency' has in most cases been omitted in column 3. AD: autosomal dominant; AR: autosomal recessive; CD: cluster of differentiation; IFN: interferon; Ig: immunoglobulin; IL: interleukin; IUIS: International Union of Immunological Societies; L: ligand; LAD: leucocyte adhesion deficiency; PID: primary immunodeficiency disease; PNH: paroxysmal nocturnal haemoglobinuria; R: receptor; SCT: stem cell transplantation.

Protocol 1

Step 1	***Rule out severe antibody deficiency and neutropenia***
Perform	Blood count and differential (check platelet volume, absolute lymphocyte, neutrophil and eosinophil counts). IgG, IgA, and IgM. IgE.
Next step	*Neutropenia:* go to protocol 3, step 2. *Agammaglobulinaemia*: go to step 4. *Hypogammaglobulinaemia*: go to step 2a. *Other*: go to step 2b

Step 2a	***Predominantly antibody deficiencies***
Hypogamma globulinaemia	*If not secondary to drugs, lymphoid malignancy, thymoma, immunoglobulin loss (urine, faeces), perform*: booster responses (tetanus; unconjugated pneumococcal vaccine if >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>4g/l) and M-proteins
Next step	Go to step 4.
Step 2b	***Predominantly antibody deficiencies or complement deficiencies***
Normal results step 1	*When positive family history or problems persist, perform*: booster responses, CH_{50} and AP_{50}, *consider*: IgG-subclasses and M-proteins; MBL, asplenia *In case of angioedema*: C1-inhibitor level, C4 during attack
Next step	*Normal results*: Wait and see. Repeat total IgG, IgA, IgM, and IgG-subclasses after 1–2 years (6 months if <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

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***
Abnormal results step 1	*Agammaglobulinaemia*: lymphocyte subpopulations (Table 4), consider lymphocyte proliferation tests (Table 4), B cell maturation analysis in bone marrow. Genetic determination of defect if possible
Abnormal results step 2	*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 CH_{50} and/or AP_{50}*: 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
Abnormal results step 3	Follow appropriate work-up guided by clinical presentation and laboratory results. Genetic determination of defect if possible

Fig. 1. Protocol 1. ANA: anti-nuclear antibody; C: complement; CD: cluster of differentiation; Ig: immunoglobulin; MBL: mannose binding lectin; PID: primary immunodeficiency. Grey 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

Fig. 2. 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. Grey shading: consultation with an immunologist is highly recommended.

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Protocol 3

Step 1	*Identify neutropenia*
Perform	Blood count and differential (absolute neutrophil count, microscopic evaluation; giant granules, bilobed nuclei, Howell-Jolly bodies); perform repeatedly in case of cyclic pattern of fever and infections (no evidence-based guidelines exist; 3 × /week for 3-6 weeks is advocated in several reviews)
Next step	*Neutropenia*: go to step 2. *Neutrophilia*: go to step 3. *Normal results*: determine IgG, IgA, IgM, CH_{50}; if normal, go to step 3; if abnormal go to protocol 1

Step 2	*Identify the cause of the neutropenia*
Isolated neutropenia	*Consider secondary causes*: drug use, autoimmunity, alloimmunity (neonate), viral infection, agammaglobulinaemia. *Perform*: autoantibodies, alloantibodies (neonate), IgG, IgA, IgM; consider ANA, C3/C4, RF, ANCA, Coombs. *If normal*: analysis of bone marrow (morphology, cytogenetic studies). Consider associated immune/metabolic disorder and appropriate tests (exocrine pancreatic function, echocardiography, brain imaging, hearing test, skin and hair analysis) Go to step 4
Pancytopenia	Analysis of bone marrow (morphology, cytogenetic studies, immunophenotyping). Collaborate with a haematologist

Step 3	*Identify defects in phagocyte function*
Perform	*Normal neutrophil count*: phagocyte function tests (Table 5). Serum IgE. Consider electron microscopy, hair evaluation. *Neutrophilia*: consider CD11b/CD18, sLeX, kindlin3 expression (flowcytometry)
Next step	*Abnormal* results: go to step 4. *Normal results*: go to protocol 1. Consider periodic fever syndromes; IgD, CRP, ESR, cytokines and urine mevalonic acid during attack; when abnormal go to step 4

Step 4	*Final diagnosis*
Perform	Determine genetic defect if possible.

Fig. 3. Protocol 3. ANA: anti-nuclear antibody; ANCA: anti-neutrophil cytoplasmic antibodies; C: complement component; CD: cluster of differentiation; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; GCSF: granulocyte–colony-stimulating factor; Ig: immunoglobulin; RF: rheumatoid factor; sLeX: sialyl-Lewis X. Grey shading: consultation with an immunologist is highly recommended.

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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	
$CD3^+$	T lymphocytes
$CD3^+/CD4^+$	Helper-T lymphocytes
$CD3^+/CD4^+/CD27^+/CD45RA^+$	Naive helper-T lymphocytes
$CD3^+/CD8^+$	Cytotoxic T lymphocytes
$CD3^+/HLA\text{-}DR^+$	Activated T lymphocytes
$CD3^+/TCR\text{-}\alpha\beta^+/CD4^-/CD8^-$	'Double-negative' TCR-$\alpha\beta^+$ T cells
$CD3^+/TCR\text{-}\gamma\delta^+$	TCR-$\gamma\delta^+$ subset of T lymphocytes
$CD19^+$ or $CD20^+$	B lymphocytes
$CD19^+/CD27^+/IgM^-/IgD^-$	Switched memory-B lymphocytes
$CD3^-/CD16^+$ and/or $CD56^+$	NK cells
(b) Determine the uptake of [^{3}H]-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: phorbol myristate 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. 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. CD: cluster of differentiation; FITC: fluorescein isothiocyanate; FMLP: formyl-met-leu-phe, a bacterial peptide; LPS: lipopolysaccharide; PMA: phorbol myristate acetate.

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Disclosure

None.

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