New Diagnostic Guidelines for Primary Immunodeficiencies

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How to improve your skills in diagnosing PID in everyday pediatric practice (in Denmark)

- Frequency & Awareness
- Diagnostic tables
- Clinical Phenotype and Presentation
- Key papers
- Initial Diagnostic approach
- Where to find help

Frequency & Awareness

- Frequency
  - Normal variations / polymorphisms
    - MBL deficiency, Selective IgA deficiency
  - Individual destinct diseases
    - unusual to extremely rare
    - Overall prevalence estimated at 1:2000
- Awareness
  - Warning signs
    - Pediatric
    - Adult

Clinical Phenotypes

- The International Union of Immunological Societies (IUIS)
- Classification & Phenotypic approach
  - Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. Front Immunol. 2011;2:54.
  - A phenotypic approach for IUIS PID classification and diagnosis: guidelines for clinicians at the bedside. J Clin Immunol. 2013 Aug;33(6):1078-87.

Table 1 | Combined immunodeficiencies.

Disease	Circulating T cells	Circulating B cells	Serum Ig	Associated features	Inheri-tance	Genetic defect/ presumed pathogenesis	OMIM number
1. T^-B^+ Severe combined immunodeficiency (SCID)							
(a) γc deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells; leaky cases may present with low to normal T and/or NK cells or Omenn syndrome	XL	Defect in γ chain of receptors for IL-2, -4, -7, -9, -15, -21	300400
(b) JAK3 deficiency	Markedly decreased	Normal or increased	Decreased	Markedly decreased NK cells; leaky cases may present with variable T and/or NK cells	AR	Defect in Janus activating kinase 3	600173
(c) IL7Rα deficiency	Markedly decreased	Normal or increased	Decreased	Normal NK cells	AR	Defect in IL-7 receptor α chain	146661
(d) CD45 deficiency*	Markedly decreased	Normal	Decreased	Normal γ/δ T cells	AR	Defect in CD45	151460
(e) CD3δ*/ CD3ε*/CD3ζ* deficiency	Markedly decreased	Normal	Decreased	Normal NK cells No γ/δT cells	AR	Defect in CD3δ, CD3ε, or CD3ζ chains of T cell antigen receptor complex	186790, 186830, 186740
(f) Coronin-1A deficiency*	Markedly decreased	Normal	Decreased	Detectable thymus	AR	Defective thymic egress of T cells and defective T cell locomotion	605000
2. T^-B^- SCID							
(a) RAG 1/2 deficiency	Markedly decreased	Markedly decreased	Decreased	May present with Omenn syndrome, expanded γ/δT cells, autoimmunity, and/or granulomas	AR	Defective VDJ recombination; defect of recombinase activating gene (RAG) 1 or 2	601457
(b) *DCLRE1C*	Markedly	Markedly	Decreased	Defective VDJ recombination,	AR	Defective VDJ recombination;	602450

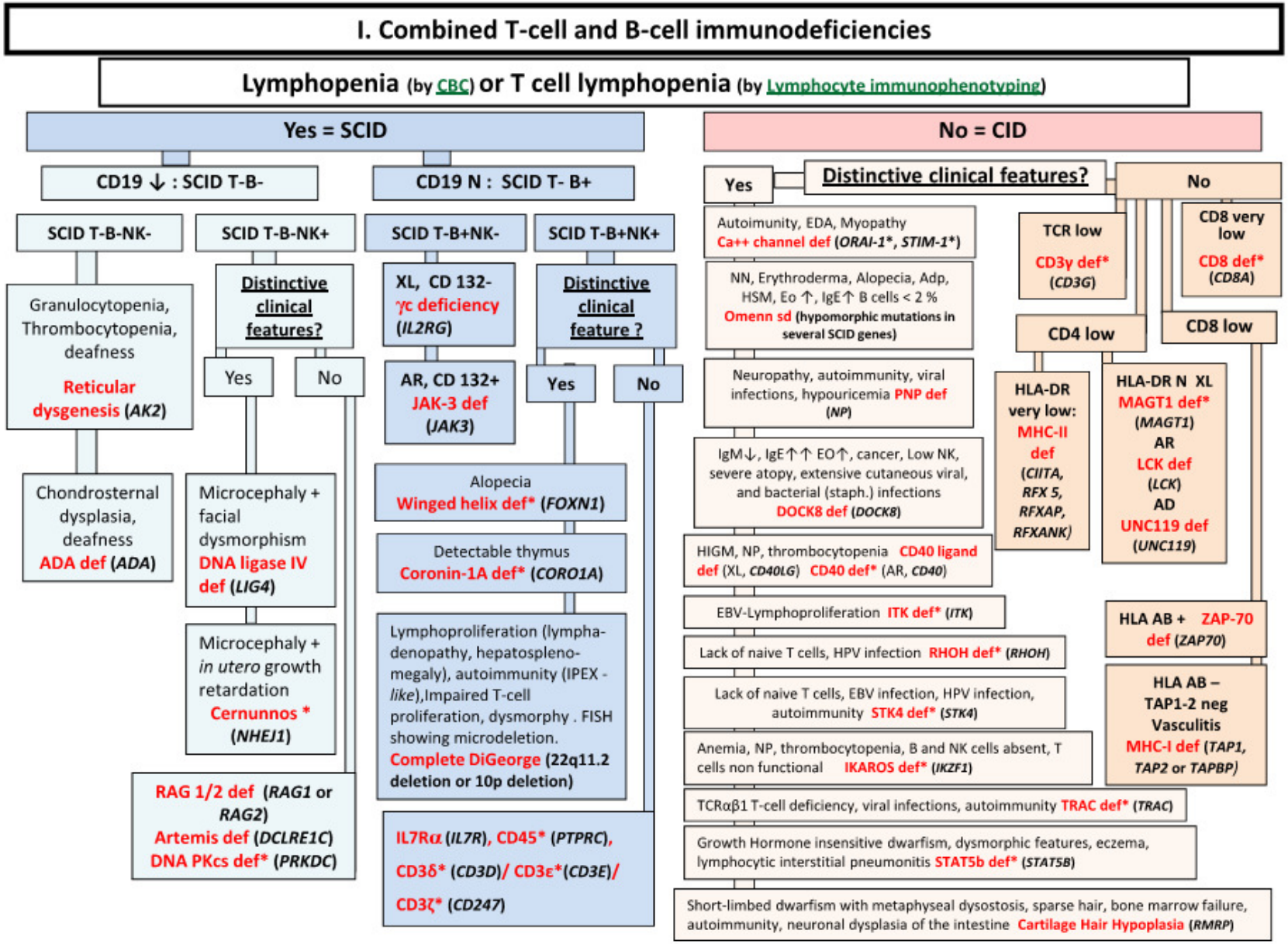

Fig. 1 Combined T- and B- cell immunodeficiencies. ADA: Adenosine Deaminase; Adp: adenopathy; AIHA: Auto-Immune Hemolytic Anemia; AR: Autosomal Recessive inheritance; CBC: Complete Blood Count; CD: Cluster of Differentiation; CID: Combined Immunodeficiency; EBV: Epstein-Barr Virus; EDA: Anhidrotic ectodermal dysplasia; EO: Eosinophils; **FISH:** Fluorescence in situ Hybridization; HIGM: Hyper IgM syndrome; HLA: Human Leukocyte Antigen; HSM: Hepatosplenomegaly; Ig: Immunoglobulin; N: Normal, not low; NK: Natural Killer; NN: Neonate; NP: Neutropenia; PT: Platelet; SCID: Severe Combined ImmunoDeficiency; TCR: T-Cell Receptor; XL: X-Linked

Table 3 | Predominantly antibody deficiencies.

Disease	Serum Ig	Associated features	Inheritance	Genetic defect/ presumed pathogenesis	OMIM number
1. Severe reduction in all serum immunoglobulin isotypes with profoundly decreased or absent B cells					
(a) BTK deficiency	All isotypes decreased in majority of patients; some patients have detectable immunoglobulins	Severe bacterial infections; normal numbers of pro-B cells	XL	Mutations in *BTK*, a cytoplasmic tyrosine kinase activated by crosslinking of the BCR	300300
(b) μ Heavy chain deficiency	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in μ heavy chain	147020
(c) λ5 deficiency*	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in λ5; part of the surrogate light chain in the pre-BCR	146770
(d) Igα deficiency*	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in Igα (*CD79a*); part of the pre-BCR and BCR	112205
(e) Igβ deficiency*	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in Igβ (*CD79b*); part of the pre-BCR and BCR	147245
(f) BLNK deficiency*	All isotypes decreased	Severe bacterial infections; normal numbers of pro-B cells	AR	Mutations in *BLNK*; a scaffold protein that binds to BTK	604615
(g) Thymoma with immunodeficiency	One or more isotypes may be decreased	Bacterial and opportunistic infections; autoimmunity; decreased number of pro-B cells	None	Unknown	
(h) Myelodysplasia with hypogammaglobuline-mia	One or more isotypes may be decreased	Infections; decreased number of pro-B cells	Variable	May have monosomy 7, trisomy 8, or dyskeratosis congenita	
2. Severe reduction in at least 2 serum immunoglobulin isotypes with normal or low number of B cells					
(a) Common variable immunodeficiency disorders	Low IgG and IgA and/or IgM	Clinical phenotypes vary: most have recurrent infections, some have polyclonal lymphoproliferation.	Variable	Unknown	

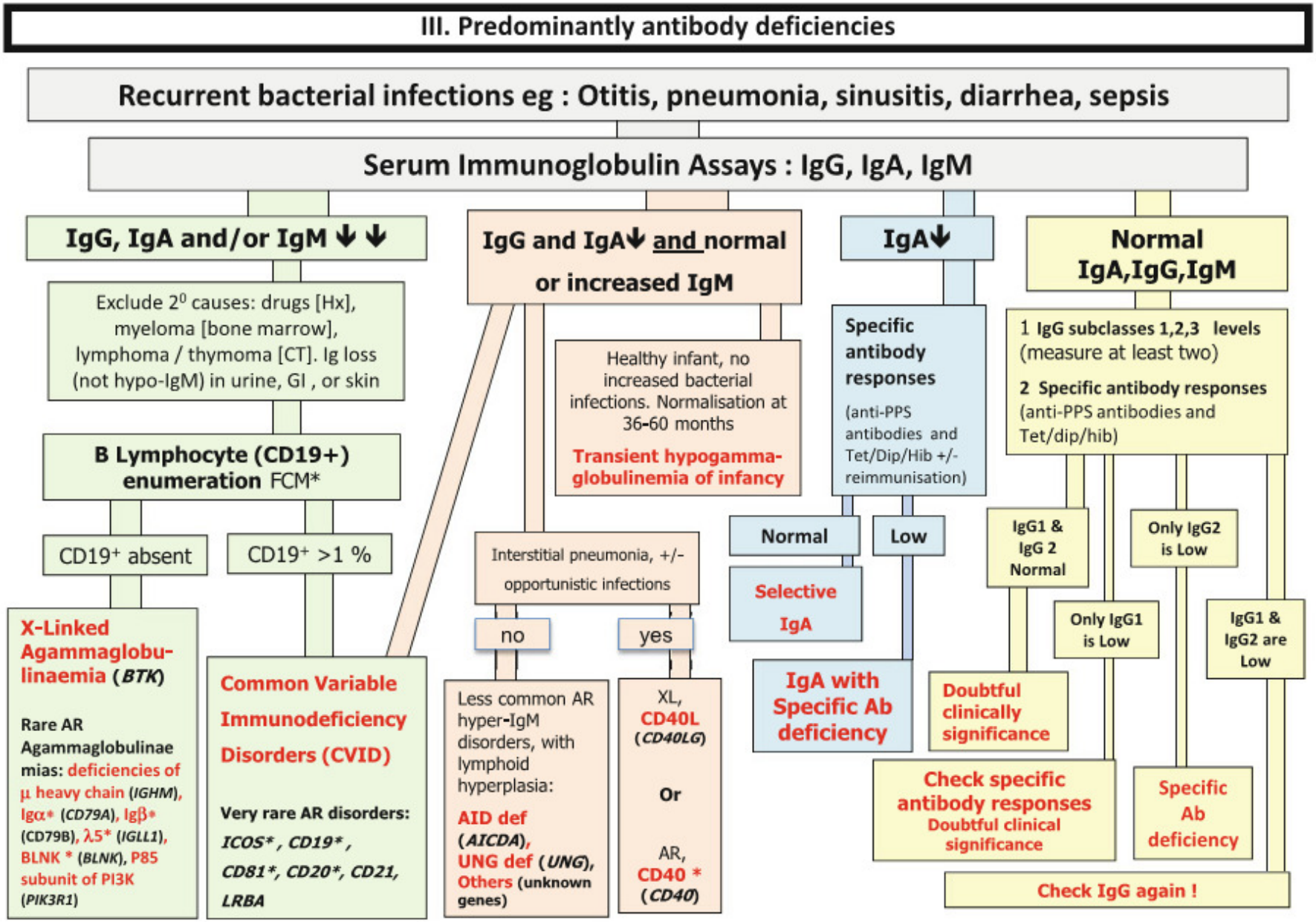

Fig. 3 Predominantly antibody deficiencies. Ab: Antibody; Anti PPS: Anti- pneumococcal polysaccharide antibodies: AR: Autosomal Recessive inheritance; CD: Cluster of Differentiation; CVID: Common Variable Immunodeficiency Disorders; CT: Computed Tomography; Dip: Diphtheria; FCM*: Flow cytometry available; GI: Gastrointestinal; Hib: *Haemophilus influenzae* serotype b; Hx: medical history; Ig: Immunoglobulin; subcl: IgG subclass; Tet; Tetanus; XL: X-Linked inheritance

Clinical Phenotypes

- European Society of Immunodeficiencies (ESID)
  - Patient-centred screening for primary immunodefici-ency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. de Vries E; European Society for Immunodeficien-cies (ESID) members.Clin Exp Immunol. 2012 Jan;167(1):108-19

Phenotypes

- Recurrent ENT & airway infections
- Failure to thrive in infancy
- Recurrent pyogenic infections
- Unusual infections or course of infections
- Recurrent inf with same pathogen
- Autoimmune or chronic inflammatory disease, lymphoproliferation
- Syndromatic disease
- Angioedema

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

Table 2. Pattern recognition gives direction to the diagnostic process.

Clinical presentation	Encountered pathogens	Special features	Non-immunological differential diagnosis	Diagnostic protocol
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	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

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.

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)
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

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.

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.

Warning signs: a time for a change?

- O'Sullivan and Cant
  - The 10 warning signs: a time for a change? O'Sullivan MD, Cant AJ. Curr Opin Allergy Clin Immunol. 2012 Dec;12(6):588-94.
  - Warning signs. Sensitivity & specificity
    - Family history
    - Iv antibiotics
    - Failure to thrive in infancy
  - Targeted Warning signs
    - Neonatal physician
    - Dermatologist
    - Gastroenterologist
    - ENT / airway specialist
    - Infectious disease specialist
    - Endocrinologist, reumatologist, etc.

Get help

- Supplementary reading
  - http://www.rigshospitalet.dk/NR/rdonlyres/378A0019-E98C-4B81-9573-DCF16BC6554A/0/Immundefekt.PDF
- Call a friend at your regional referral center
  - Pediatric dept
  - Clinical Immunology dept