UZ LEUVEN | LABORATORIUMGENEESKUNDE

CAT
Critically Appraised Topic

Title: Primary Immunodeficiencies: towards a rational testing approach

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CLINICAL BOTTOM LINE

Primary immunodeficiencies (PID) in children are rare, but early diagnosis is important for appropriate treatment and favorable outcomes. The threshold for thinking of PID should therefore be low, and clinical alarm signs have been well-defined. However, the best strategy for laboratory testing once a PID is clinically suspected, is unclear. In Imelda hospital, the most commonly ordered laboratory tests for suspected PID are full blood count, quantification of immunoglobulins (Ig), measurement of IgG2 (an IgG subclass) and flow cytometry for classification of lymphocytes into B-cells, T-cells and CD4/CD8 positive cells. Less frequently, humoral immune responses to pneumococcal vaccination are assessed. However, it is unclear which clinical signs or symptoms prompt the clinicians to order either of these tests, and whether the diagnostic strategy is optimal for prompt diagnosis of PID. For this CAT, we aim to provide the clinician with evidence-based criteria when to suspect PID and the appropriate testing strategy in these cases, based on clinical presentation and prevalence of these disorders, also taking into account other possible diagnoses. Four "testing protocols" were designed, based on literature review and expert recommendations. These protocols consist of first line, second line and third line tests. First line tests are full blood count, quantification of immunoglobulins and, depending on the clinical presentation, flow cytometry to differentiate lymphocytes and tests to exclude or confirm the presence of other conditions. Second and third line tests are more specialized immunological tests and should only be ordered in case of aberrant first line test results and/or strong clinical suspicion.

CLINICAL/DIAGNOSTIC SCENARIO

Primary immunodeficiencies (PID) are a group of disorders, defined as inborn, genetic errors of immunity, as opposed to secondary immunodeficiencies, which are acquired throughout life (*e.g.* HIV, treatment with chemotherapy). The most frequent presentation of an immunodeficiency is **recurrent** infection, **unusually severe** infections or the occurrence of infections with **rare and atypical pathogens**. However, it has become increasingly clear that many of the PIDs are also associated with immune dysregulation and auto-immune disorders (1). Currently, 430 inborn errors of immunity have been identified, of which 64 in the last two years (2). All of these disorders are exceedingly rare; but as a group, the estimate for PID prevalence range from 1/20.000 to 1/1.200, depending on the source and method of estimation (1,3,4). Most national registry data probably underestimate the true prevalence of PID, especially in developing countries (4).

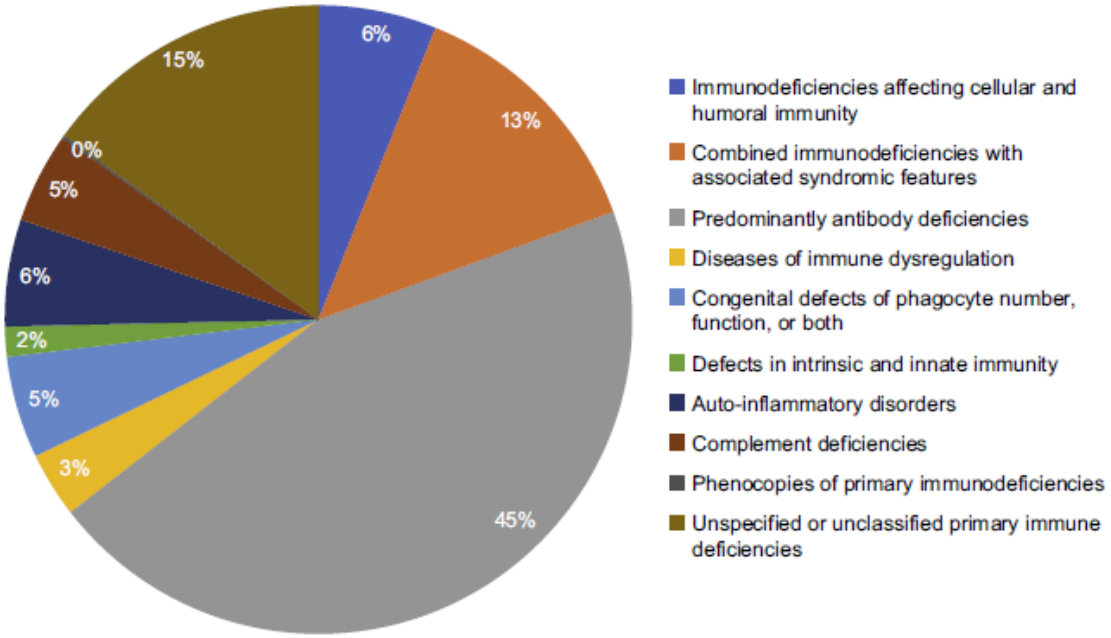

Figure 1: Relative frequency of the different classes of PID (1)

As early diagnosis is usually associated with better long term outcomes (5,6), alarm symptoms which should prompt the clinician to think of PID were developed by the Jeffrey Model Foundation in 1994 (see box 1). These are generally well-known among pediatricians. However, these signs are based on expert opinion and have not been well validated. Their diagnostic performance has been evaluated in a few retrospective studies and appears to be rather poor (7–9). As a consequence, some authors have suggested additional "alarm signs" to consider PID, including non-infectious presentations such as auto-immunity (7,10,11).

Secondly, when a PID is suspected clinically, there are no clear guidelines on how to proceed to exclude or confirm the diagnosis. In addition, most children who meet the Jeffrey Model Foundation criteria are normal children, and therefore it is unclear how clinicians should proceed when first-line or second-line tests come back negative. The decision whether to continue investigations is especially challenging in children suspected of mild immune deficiencies, as both clinical presentation and laboratory test results may be borderline or ambiguous.

Jeffrey-Modell foundation: 10 warning signs of PID

If a child presents with **two or more** of the following warning signs, think of PID:

- Four or more ear infections within one year
- Two or more serious sinus infections within one year
- Two or more months of antibiotics with little effect
- Two or more pneumonias within one year
- Failure of an infant to gain weight or grow normally
- Recurrent, deep skin or organ abscesses
- Persistent thrush in mouth or fungal infection of skin
- Need for IV antibiotics to clear infection
- Two or more deep-seated infections, including sepsis
- A family history of PID

Box 1: the Jeffrey-Modell foundation warning signs

Current test strategy in Imelda hospital

Imelda hospital is a medium-sized secondary care hospital in Belgium, with 624 inpatient beds, of which 20 pediatric. When clinicians suspect a PID in children, the "second-line" tests (after full blood count with white blood cell differentiation and immunoglobulin quantification) most commonly ordered at the moment are IgG2 subclass, CH50 (complement hemolytic assay), T/B/NK cell differentiation and vaccination antibody response (see Table 1).

AP50 (alternative pathway hemolytic assay) and respiratory burst assay are ordered sporadically (both 2 times since 2019).

From 2019 until now, IgG2 was measured 274 times, of which 228 tests in children. Mean age of these children was 3.7 years, median age was 2.2 years. Ordering of this test led to one clinical diagnosis of subclass deficiency over the last two years, however in this case the subclass deficiency was not considered clinically relevant, as vaccine response was within normal limits (expert opinion in University Hospital Antwerp). CH50 assays were ordered for 32 children; no complement deficiency was detected in these patients. T/B/NK cell differentiation was ordered 22 times in children from 2019 until now. Mild lymphopenia was detected in 3 of these children, but T/B/NK differentiation was normal and a PID was not detected. Pneumococcal vaccine challenges were ordered in 6 children; in 2 of these patients, the test was not performed as only 1 (pre-vaccination) test was ordered. One of the 4 patients in which the test was performed, was subsequently diagnosed with a specific antibody deficiency in combination with IgA deficiency.

Table 1: Second-line test ordering in Imelda hospital for diagnosis of primary immunodeficiency according to age and sex of patient.

		Number of IgG2 tests (% of total)	Number of TBNK flow cytometry (% of total)	Number of CH50 assays (% of total)	Number of pneumococcal vaccine challenge tests (% of total
Sex	Male	173 (63.1%)	24 (46.2%)	29 (74.4%)	7 (53.8%)
	Female	101 (36.9%)	28 (53.8%)	10 (25.6%)	6 (46.2%)
Age category	0-5 years	181 (66.1%)	18 (23.7%)	27 (69.2%)	6 (46.2%)
	5-10 years	22 (8.0%)	2 (2.6%)	4 (10.3%)	0
	10-15 years	21 (7.7%)	2 (2.6%)	0	0
	15-18 years	4 (1.5%)	0 (0%)	1 (2.6%)	0
	> 18 years	46 (16.8%)	54 (71.3%)	7 (17.9%)	7 (53.8%)
Total		274	76	39	13

In general, it remains unclear which tests should be ordered for which symptoms, and how different testing strategies lead to either under- or over-testing. The low "diagnostic yield" of IgG2, the most commonly ordered test, may indicate the need for optimalisation of the diagnostic strategy.
The general aim of this CAT is therefore to elucidate "optimal" testing protocols for diagnosis of PID, with the focus on the secondary care setting.

QUESTION(S)

1) *What are the different clinical and demographic features of primary immunodeficiencies?*
2) *How to evaluate children with recurrent infections? When is further testing required?*
3) *Which tests should be ordered as a first assessment? Based on the results of these tests, how should the clinician proceed?*

SEARCH TERMS

1) *MeSH Database (PubMed): MeSH term: "Primary Immunodeficiency Diseases/diagnosis"[MeSH]*
2) *PubMed:*
    a. *"primary immunodeficiency" AND (diagnosis OR approach OR algorithm)*
    b. *"recurrent infections" AND children*
3) *International organizations:* International Union of Immunological Societies (IUIS) Expert Committee; Immune Deficiency Foundation
4) *UpToDate Online: "recurrent infections", "primary immunodeficiency"*

RELEVANT EVIDENCE/REFERENCES

1) *Guidelines and Recommendations (most recent topics on top))*

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2) *Systematic Reviews and Meta-analyses*

None

3) *Reviews*

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1. Clinical characteristics of primary immunodeficiencies

Overview and classification of primary immunodeficiencies

PIDs are usually classified according to the part of the immune system that is deficient and/or according to the most typical clinical presentations. Relative frequency of the different classes of PID is given in Figure 1. The following classes of PID are identified by the latest classification by the International Union of Immunological Societies (IUIS) Expert Committee (2):

1. Antibody deficiencies

This is the most prevalent group of PIDs, comprising around 45% of all PIDs (1). In these disorders B-cells are usually decreased or absent, and immunoglobulin levels are low to non-detectable. The clinical picture of antibody deficiencies is that of recurrent respiratory infections, severe invasive bacterial infections and recurrent infections with encapsulated bacteria. Chronic diarrhea due to rotavirus infections, *Giardia lamblia* or *Campylobacter* is also common in these conditions. Bronchiectasis is both a cause and a result of recurrent respiratory infections, therefore presence of bronchiectasis in children is not a final explanation for recurrent infections and should prompt investigations for antibody deficiencies.

- The most serious forms of antibody deficiencies are characterized by agammaglobulinemia and absence or severe decrease of B-cells.
- Less severe but much more prevalent is Common Variable Immune Deficiency (CVID). No genetic mutations have been identified as yet for this condition, which is defined by low levels of IgG and IgA and/or IgM, and an abnormal antibody response to specific antigens (usually measured by pneumococcal vaccine antibody response). It generally presents at adolescence or in adulthood (the oldest patient diagnosed with CVID was 92 years old!) (12).
- HyperIgM syndrome is characterized by low levels of IgG and IgA but (very) high levels of IgM, due to a lack of B-cell maturation.
- Selective IgA deficiency is characterized by total absence of IgA (to be measured with radial immunodiffusion). It is actually a very common condition, occurring in 1/500 individuals, many of whom do not present with an immune deficiency.
- IgG subclass deficiency is also usually asymptomatic. A minority can present with poor humoral vaccine response and recurrent infections. The combination of an IgG subclass deficiency and IgA deficiency is often a precursor to CVID (13).
- Specific antibody deficiencies are defined by normal levels of immunoglobulins, but reduced humoral vaccine response.
- To complicate diagnosis in children, physiological hypogammaglobulinemia occurs between 3 and 6 months of age, when the maternal antibodies decrease and the production of immunoglobulins is still low (14). This is expected and not clinically significant. Transient hypogammaglobulinemia of infancy (THI), on the other hand, is diagnosed when this hypogammaglobulinemia (mostly IgG and IgA) persists between 5 and 24 months of age (15). This condition may be associated with recurrent infections, but most patients are asymptomatic and there is usually a normal ability to produce antibodies to vaccine antigens (2,14). Moreover, the condition is by definition self-limiting and normal immunoglobulin levels are expected before the age of 4 years.

2. Combined cellular & humoral deficiencies

When both cellular and humoral immunity are affected, we speak of combined deficiencies. The clinical picture of CID is usually that of infections with unusual pathogens, recurrent infections with yeast, fungi or viruses, failure to thrive and/or persistent diarrhea. This class accounts for approximately 6% of PIDs,

and comprises the syndromes leading to a severe combined immunodeficiency (SCID) and other, less profound combined immunodeficiencies (CID) (1).

- SCID is rare (1/58.000 births) but has high mortality without treatment, and earlier treatment (usually allogenic stem cell transplantation) has a better prognosis than later treatment (5,6). Therefore, early diagnosis is key for these disorders. In SCID, T-cells are usually very low to absent, while B-cell numbers are variable. Some disorders are characterized by very low B-cell counts (SCID T-B-), whereas others have normal B-cells but decreased B-cell function (poor specific antibody responses) and/or low immunoglobulins (SCID T-B+). Newborn screening for SCID by detection of T-cell receptor excision circles (TREC) is practiced in the USA and some European countries (16). The number of conditions leading to combined deficiencies is high and many possible combinations of T-cell, B-cell and immunoglobulin abnormalities have been described.
- In some of the less severe CID syndromes, absolute numbers of T-cells and B-cells are normal but their function is decreased, with immunoglobulins ranging from low to normal levels. These disorders are evidently the most difficult to diagnose.
- Further complicating diagnosis of CID is the observation that infants with SCID can have maternal T cell engraftment, leading to normal numbers of T-cells but with abnormal function, often resulting in autoimmune cytopenias or graft-versus-host disease.

3. Combined immunodeficiencies with associated or syndromic features

These genetic disorders are primarily characterized by their associated and syndromic features, and their diagnosis is usually prompted by these syndromic features and not because of the occurrence of frequent or atypical infections. Examples are the DiGeorge (velocardiofacial) syndrome, characterized by facial abnormalities, conotruncal cardiac malformation, velopalatal insufficiency and intellectual disability, and ataxia-telangiectasia, which is characterized by ataxia, telangiectasias especially of the sclerae and pulmonary infections. Together, these disorders account for 13% of immunodeficiencies (1). For diagnosis and management of CID with syndromic features, referral to clinical geneticists is advised.

4. Phagocyte defects

Phagocyte defects are present in 5% of PIDs (1). These disorders can be roughly divided in two groups: quantitative defects (congenital neutropenia, bone marrow failure) and functional defects, such as defects of white blood cell motility (*e.g.*, leukocyte adhesion deficiencies) and defects of respiratory burst. The most common among these disorders is chronic granulomatous disease (CGD), which is a respiratory burst defect. These disorders predispose to recurrent pyogenic infections, deep-seated granulomatous skin, bone, lung or liver infections and fungal infections. Poor wound healing and delayed umbilical cord detachment are other features of these conditions.

5. Immune dysregulation syndromes

This class of disorders is characterized mainly by the presence of auto-immune disorders (such as auto-immune cytopenias, inflammatory bowel disorders and endocrine auto-immune diseases), hemophagocytic lymphohistiocytosis and/or increased risk of lymphoproliferative diseases such as lymphoma. Many of these disorders are also associated with increased susceptibility to (chronic) EBV infection and EBV-related lymphoproliferation. Together, these disorders are responsible for 3% of all PIDs (1).

6. Auto-inflammatory syndromes

As suggested by the name, these syndromes are characterized mainly by auto-immune and auto-inflammatory symptoms. These disorders, accounting for 6% of PIDs, can be divided into type 1 interferonopathies, defects affecting the inflammasome (such as familial Mediterranean fever) and other, non-inflammasome-related conditions characterized by auto-inflammation (1,2).

7. Complement deficiencies

Complement deficiencies are responsible for 5% of PIDs and are mainly associated with bacterial infections (1). Presentation depends on the complement factor that is missing or deficient. For example, C3 is an important opsonin, therefore its deficiency infers increased susceptibility to encapsulated bacteria (e.g., *Streptococcus pneumonia*), for which opsonization is the primary host defense. C1, C4 and C2 deficiency are more common but less severe, as C3 can still be activated by the alternative pathway (17). Deficiencies of the terminal factors (C5-C9) and the alternative pathway (factor I, factor H, factor D) do not lead to pneumococcal infections, but predispose to recurrent invasive *Neisseria* infections. Of note, complement deficiencies are also strongly associated with systemic lupus erythematosus (especially C1, C2 and C4 deficiency), while C3 deficiency also leads to membranoproliferative glomerulonephritis. Factors from the alternative complement pathway are also implicated in complement-mediated thrombotic microangiopathy. The lectin pathway seems less important for the normal functioning of the immune system, as many of the deficiencies described in this pathway (such as mannose-binding lectin deficiency) are not strongly associated with increased susceptibility for infections (present in 5% of the population!) (13).

8. Innate immune defects

Innate immunity defects are rare (2% of PID) and consist of either Toll-like receptor (TLR) deficiencies or defects in the interferon (IFN) and interleukin (IL) pathways, particularly those associated with IFN-gamma, IL-12, IL-17 and IL-1. Depending on the defect, different infection patterns are seen. For example, recurrent infections with intracellular bacteria such as atypical Mycobacteria and *Salmonella* species and severe viral infections are seen typically with defects of the IL-12 & IFN-gamma pathways. HSV encephalitis in neonates is indicative for a TLR-3 receptor deficiency. Other TLR deficiencies, or defects along their pathways, predispose for bacterial infections. Usually, innate immune defects present early in life but improve with age (18).

There is considerable overlap between the different categories of PID; as an example, hyper IgE syndromes are characterized by very high levels of IgE, but low levels of specific antibody production, suggestive for a specific antibody deficiency. However, in most Hyper IgE syndromes, syndromic features are present (*e.g.*, Job syndrome, with distinctive facial features), which would point towards the category of combined immunodeficiencies with associated or syndromic features. In terms of infection susceptibility, however, there is an important overlap with phagocyte defects, with high propensity to fungal and pyogenic infections and decreased neutrophile function (13). Similarly, STAT1 gain of function mutations lead to deficient development of IL-17 producing T-cells, resulting in chronic mucocutaneous candidiasis and viral infections, but the syndrome is also associated with auto-immunity. Depending on the source, it is classified under “Immune dysregulation syndromes” or “Defects in intrinsic and innate immunity” (2,19). As many aspects of the immune system influence each other, overlap in presentation is to be expected.

A table giving a comprehensive overview and summary of the different classes of immunodeficiency and their most important laboratory and clinical features can be found as attachment 1.

Recent publications expand the diagnosis of PID to all congenital conditions that can present with reduced immunity to infections, and not just those with defects in the immune system. Conditions such as cystic fibrosis (a chloride channel defect leading to reduced pulmonary clearing of pathogens) and sickle cell disease (a hematological condition leading to functional asplenia, which in turns predisposes to infections with encapsulated organisms) are thus also included in the definition of PID (20). Of course, these conditions should be considered in the differential diagnosis of children presenting with recurrent infections, but for the sake of this overview we have not included them as primary immune deficiencies.

2. Assessment of the child with recurrent infections

Due to the importance of early diagnosis, criteria for clinicians to think of PID have been established by the Jeffrey Modell Foundation. The "10 warning signs of PID" are widely taught among clinicians and are presented in box 1 (see above). These alarm symptoms are based on expert opinion and their diagnostic performance appears to be rather poor; in retrospective evaluations, sensitivity of the alarm signs in children was reported to be between 62 and 64% (7–9). Specificity is even lower (23% and 48-56% for presence of 1 and 2 alarm signs respectively) (7–9), but this is less problematic as alarm signs are meant as a first screening tool.

Some retrospective studies (7,8,21,22) and one, very small, prospective study in children < 6 months (23) have looked at the predictive value of the different warning signs and other signs. The (significant) associations these studies found are presented in Table 2. Failure to thrive and recurrent pneumonia are signs significantly associated with PID in more than one study; family history of PID was the most important predictor of PID by far in the study by Subbarayan et al. Need for IV antibiotics was significantly associated with PID overall in one study (7) and with neutrophil PID (but not with PID overall) in another (21). As these studies were performed in children already suspected of PID and recurrent upper respiratory tract infections are relatively common in normal children, it is not surprising that upper respiratory tract infections appeared to be inversely associated with diagnosis of PID, although the results for otitis media are conflicting between studies. For atopy as well, conflicting results were found between studies. Atopy or allergic conditions may be more common in children with PID, but can on the other hand also be an alternative explanation for signs and symptoms of recurrent infections (see below).

Table 2: Association of clinical signs, among which the warning signs, with diagnosis of PID. Only signs/symptoms explicitly mentioned in each study are filled in this table. Some non-significant associations may not have been explicitly mentioned in each study

	Subbarayan et al, 2011 (21)	**Bjelac et al, 2019** (7)	**Galal et al, 2019** (23)	**MacGinnity et al, 2011** (8)	**Bahrami et al, 2020** (22)
Number of PID cases	430	115	26	32	21
Number of controls	133	2161	24	109	179
Most common PID diagnosis	SCID	DiGeorge syndrome	SCID	Transient hypogammaglobulinemia of infancy	IgA deficiency
Association of Jeffrey Model Foundation alarm signs with PID (expressed in odds ratio [OR]; OR > 1 = positive association; OR < 1 = negative association)					
Family history of PID	18 p < 0.01	-	-	NS *	-
(Recurrent) pneumonia	NS	2.9 p < 0.001	5.5 p < 0.01	NS *	NS
Failure to thrive	9 p < 0.01	2.1 p < 0.001	-	NS *	-
Need for IV antibiotics	NS	2.1 p < 0.001	-	NS *	-
Serious bacterial infections	NS	4.8 p < 0.001	-	NS *	-
Recurrent otitis media	0.5 p < 0.01	1.5 p < 0.001	-	NS *	NS
Recurrent sinusitis	0.3 p < 0.01	-	-	NS *	NS
Fungal infections	-	-	6.8 p < 0.01	-	-
Association of other signs with PID					
Auto-immunity	-	3.2	-	-	-

		p = 0.0012			
Atopy or allergy	-	0.3 p < 0.01		4.65 p = 0.011	5.03 p = 0.03
Lymphopenia	-	-	15 p < 0.01	-	-
> 2 months of oral antibiotics with little effect	14 p < 0.01	-	-	-	-
Parental consanguinity	-	-	Is said to be associated in the study but no exact figures were given	-	2.68 p = 0.02
Vaccine adverse effects	-	-	-	-	9.31 p = 0.03

* No significant associations were found in this study, but the authors pointed out that this was most likely due to small sample size

Subbarayan et al suggested their own flow-chart for evaluation of children suspected of PID, based on their findings (Figure 2).

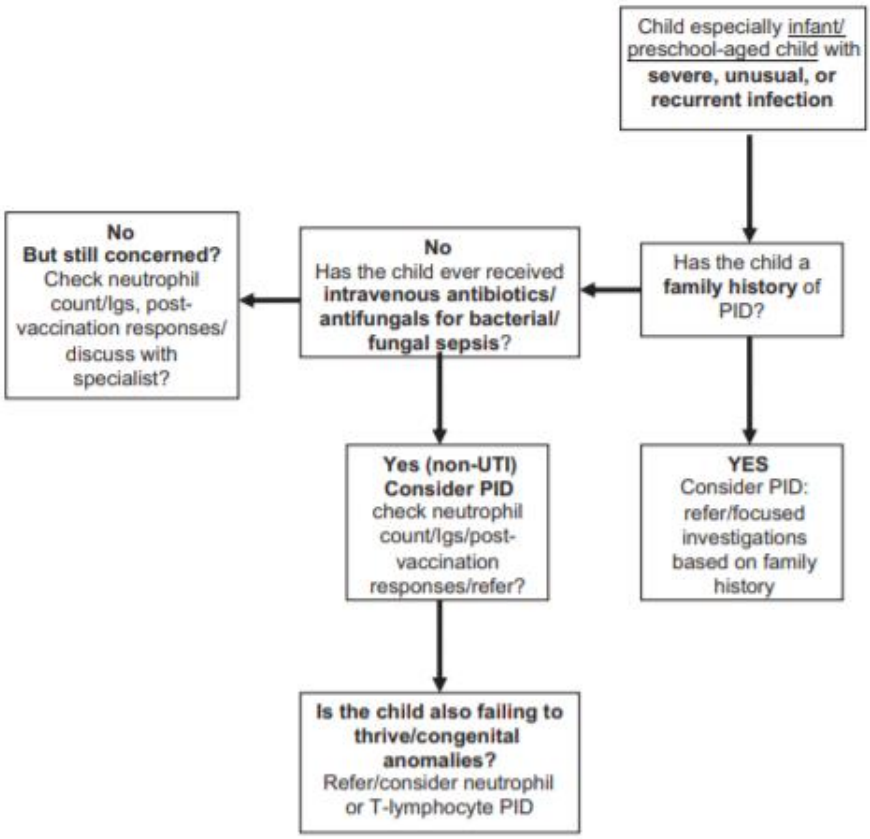

Figure 2: Diagnostic algorithm according to Subbarayan et al, 2011 (21)

As sensitivity of the "alarm signs" is not optimal, some authors have identified additional alarm symptoms. Carneiro-Sampaio et al made a list of additional symptoms which should prompt investigations for PID in children (box 2) (11). Immune dysregulation and autoimmunity, for example, are not included in the warning signs but are relatively common presentations of PID (1).

Another common presentation of PID is recurrent ENT- and airway infections. These infections are however also very common among children without an underlying immune deficiency, as demonstrated in studies which found negative associations with diagnosis of PID (Table 2). The diagnostic challenge often lies in making the distinction between children with mild PID and "normal" children with frequent infections. Benign causes for recurrent infection should therefore be considered before putting into motion the algorithms for diagnosis of PID.

Additional warning signs

- Infections by unusual micro-organisms or unusual locations
- Complications of living vaccines
- Chronic diarrhea
- Non-healing wounds
- Extensive skin lesions
- Persistent lymphopenia (< 1500/µL in children > 5 years, < 2500/µL in younger children)
- Unexplained auto-immunity or fever
- Granulomas
- Lymphoma in children
- Syndromic features
- Severe hypocalcemia

Box 2: additional warning signs for PID (11)

Causes of recurrent infections in children:

- It is estimated that 50% of these children do not have any underlying condition. Recurrent respiratory tract infections are very common in little children, and even more so when attending daycare (which, for very small children, is a more common practice in Belgium than in most other countries). A "normal" child has 4-8 respiratory infections per year *on average* (24,25). This means some children may have up to 10-12 infections per year, even when nothing is wrong with their immune system. However, most of these infections are viral, do not require antibiotics or admission and the child's weight and development is normal otherwise.
- 30% of recurrent infections are due to atopy. Atopic children (predisposed to allergic conditions) frequently have cough and wheezing due to airway hyperreactivity; however, recurrent or severe upper airway infections are also more common in these children (26). The presence of atopic signs such as eczema does not exclude a PID; some PIDs are associated with allergic conditions and eczema or skin rash is a common clinical feature of many PIDs!
- In 10% of children with recurrent infections, another chronic disease or anatomical defect is present. It is therefore important to keep other conditions in mind, such as cystic fibrosis, congenital heart disease, reflux, celiac disease or anatomical defects (especially when the recurring infections always affect the same anatomical site). In general, PID should only be suspected when the recurrent infections involve at least two different anatomical sites (27,28). Some authors with broader definitions of PID would also include these other disorders in the PID definition (20); we agree that their diagnosis or exclusion should be part of the diagnostic workup.
- An estimated 10% of children with recurrent infections suffer from PID (29).

As mentioned, the presence of other conditions does not preclude a PID! Congenital heart disease, anatomical defects, skin disorders, auto-immune and allergic conditions are all associated with PID.

Clinical presentations of PID can be subdivided in typical presentations, partly overlapping with the IUIS classification (Table 3).

Table 3: Common clinical presentations of PID

Clinical presentation	Overlap with classification
Recurrent ENT- and airway infections (including bronchiectasis)	Predominantly antibody deficiencies Complement 1,2,4 deficiencies
Recurrent invasive infections with encapsulated organisms	Predominantly antibody deficiencies Complement deficiencies (C1-4)
Failure to thrive from early infancy, including intractable diarrhea	Combined immunodeficiencies
Recurrent pyogenic infections (including granulomatous inflammation, poor wound healing)	Congenital defects of phagocyte number or function
Unusual infections or unusually severe infections	Combined immunodeficiencies Defects in innate immunity
Recurrent infections with the same type of pathogen	Defects in innate immunity Complement 5-9, alternative pathway deficiencies
Auto-immune or chronic inflammatory disease; lymphoproliferation	Diseases of immune dysregulation Auto-inflammatory disorders

Adapted from Stiehm's Immune Deficiencies, 2nd edition, 2020; Chapter 1: Common presentations and diagnostic approaches (1)

3. Test strategy for diagnosis of PID

As PID are rare diseases, "evidence-based" testing strategies are difficult to test. Most of the recommendations on PID are expert opinions. The test strategy outlined below is based on the recommendations of de Vries et al, 2012 (30), Stiehm's Immune Deficiencies, 2nd edition (Chapter 4) (31), Diagnostic & Clinical Care Guideline of the Immune Deficiency Foundation (18), Tavakol et al, 2019 (28) and Uptodate (32–34).

Depending on the clinical presentation, four different testing protocols are proposed.
These protocols are outlined below and are divided into first line, second line and third line tests, depending on the strength of clinical suspicion and results of first line and second line tests respectively. If first line test results are normal, clinical presentation is still compatible with a normal immune system (see paragraph 2) and the child is generally well, second line tests are probably not necessary and a wait-and-see approach is recommended. It is important to stress that investigations for primary immunodeficiencies are preferentially sampled **between** infectious episodes, as the presence of infections can influence many of the test results. In case mild abnormalities are found, it is advised to first repeat the test before jumping to conclusions and ordering second/third line tests.

Table 4: Suggested test strategy by clinical presentation

Clinical presentation	**Protocol**
Recurrent ENT- and airway infections (including bronchiectasis)	Protocol 1
Recurrent invasive infections with encapsulated organisms	Protocol 1
Failure to thrive from early infancy (including intractable diarrhea)	Protocol 2
Recurrent pyogenic infections (including granulomatous inflammation, poor wound healing)	Protocol 3
Unusual infections or unusually severe infections	Protocol 2 Protocol 4
Recurrent yeast/fungal infections	Protocol 2 Protocol 3 Protocol 4
Recurrent infections with the same type of pathogen	Protocol 4

The specimen is indicated in the first column with the following abbreviations:

- E = full blood EDTA
- S = serum
- H = full blood lithium heparin (without gel!)
- P = heparin plasma (with or without gel), after centrifugation
- U = urine

The color of the test indicates whether it is a first- (green), second- (yellow) or third-line (dark orange) test.
The criteria for second/third line test indicate when it is useful to order these tests. Of course, these protocols are only indicative. When there is a serious suspicion of PID, consultation with a specialized Immunology department is indicated.
The column “Information about test” pertains to practical information on sampling and time frames for sampling. For some tests, prior appointment and/or contact with the Immunology department of the referral hospital is needed.

First line tests
Important first line tests in nearly all protocols are a full blood count with white blood cell differentiation, and immunoglobulin levels. (Repeated) lymphopenia, especially in young children, is always worth further investigation to exclude SCID. Age-adapted reference values should be used, as children have higher mean lymphocyte values than adults. Normal or even elevated lymphocyte counts do not rule out “leaky” SCID (Omenn syndrome) or mother’s lymphocyte engraftment. When neutropenia is present, further investigations from protocol 3 should be followed regardless of clinical symptoms. A peripheral blood smear can occasionally be informative; cytoplasmic granules in leukocytes can point towards Chediak-Higashi syndrome, whereas Howell-Jolly bodies in erythrocytes can be a sign of asplenia. Small platelets and thrombocytopenia are suggestive of Wiskott-Aldrich syndrome (35).
Hypogammaglobulinemia can point towards a PID, but can be secondary to other causes as well, such as protein loss due to enteropathy or renal diseases. These should evidently be excluded. As mentioned above, low immunoglobulin levels in infants are not abnormal, and age-adapted reference ranges should always be used.
Anemia, thrombopenia or pancytopenia are always important findings and may warrant referral for bone marrow aspiration and biopsy. Auto-immune cytopenias are also described in a number of PIDs.

Second line tests

- **T/B/NK cell flow cytometry**: the relative and absolute numbers of CD19 positive cells (B-cells), CD3 positive cells (T-cells) and CD56 positive cells (NK-cells) can be distinguished with flow cytometry. Moreover, subclassification of T-cells into CD4+ and CD8+ cells can be done. This test is a first line test in case of clinical signs and symptoms suggestive for a combined immunodeficiency (protocol 2).

- **IgE measurement** is mainly important in diagnosis of hyperIgE syndromes, and levels > 1000 IU/ml are a reason for concern (Diagnosis & Clinical Care guideline). When there is a clinical suspicion of hyperIgE syndromes (HIES), the HIES scoring system can be used (attachment 2) to assess probability of a HIES. Levels of IgE decline with age in HIES, therefore in adults, a normal IgE can occur in these patients and does not exclude HIES (36). Although not a diagnostic criterion, undetectable IgE is often seen in CVID (37).

- **Pneumococcal polysaccharide antibodies**: this test measures antibodies against several pneumococcal serotypes before and after vaccination with Pneumovax 23. This is an important test to assess function of the humoral immune system. Polysaccharide antigens can be used as they do not elicit an effective T-cell response. It is important to measure antibodies only to those serotypes that are not included in the currently administered vaccines in childhood, such as Prevenar.
  Interpretation of the results is not straightforward; possible criteria to speak of an efficient vaccine response are an absolute antibody increase of at least 0.3 mg/L of > 50% of serotypes tested (> 70% for adults), a two- to fourfold increase in antibody titers to > 50% of serotypes tested (> 70% for adults) or an absolute antibody concentration of at least 1.3 mg/L for > 50% of serotypes tested (> 70% for adults) (38). Interpretation is best discussed with the Immunology department of the referral hospital. Another problem with the pneumococcal vaccine challenge is the high prevalence of vaccination with a conjugated pneumococcal vaccine in childhood, and the possibility of exposure to S. pneumoniae serotypes in vivo, which could affect the response to the polysaccharide vaccine. An alternative vaccine that shows promise for the diagnosis of humoral immune deficiencies is the vaccine against typhoid fever (39,40). These vaccines have the advantage that in a Western population, probability of pre-vaccination exposure to the *Salmonella* Typhi antigens is low.

- **Complement tests**: to assess complement deficiencies, two assays are generally advised: the CH50 complement hemolytic assay, which assesses the classical complement pathway, and the AP50 pathway, which assesses the alternative complement pathway. Both assays are based on quantifying the amount of serum needed to lyse 50% of a sample of erythrocytes. When both assays are low, this points to defects in the shared terminal pathway, which is constituted by complement factors C5-C9. Whether both assays should be ordered simultaneously or subsequently when a complement defect is considered, is subject to expert opinion and opinions seem to differ (30,32,41). Because defects in the "early" versus the alternative and "late" factors of the complement system usually present differently, and hemolytic assays are time-consuming and expensive, we have chosen for the more targeted approach as also recommended by Uptodate (see protocol 1, protocol 4) (32). Hemolytic assays are also known to produce variable results between laboratories (41). Methods based on enzyme-linked immunoassays (ELISA) have been developed for all three complement pathways and could be an alternative to the hemolytic assays (41).

- **ESR, CRP, ANA, RF, C3/C4, ANCA**, **immunoglobulins**: these tests are advised to exclude secondary causes of neutropenia (30). CVID, for example, is often associated with auto-immune cytopenias. Other auto-immune diseases, unrelated to PID, can also cause neutropenia.

- **Vitamin B12, folic acid:** deficiencies in these vitamins can cause the well-known megaloblastic anemia (sometimes with neutropenia), but is also associated with hypogammaglobulinemia and decrease in cellular immunity.

- **Copper & ceruloplasmin:** to exclude secondary cause of neutropenia
- **CD11b/CD18 expression:** this test can diagnose LAD1 disorder (leukocyte adhesion defect) due to absence of the CD18/CD11 integrins, which act as adhesion molecules.

- **DHR oxidation:** this assay assesses oxidative burst of neutrophils (giving an indication of phagocytosis and "killing" function of granulocytes) by detecting changes in fluorescence properties of dihydrorhodamine using flow cytometry (42).

Third line tests

- **IgG2 testing** is probably only useful in those cases where immunoglobulins are normal but infections persist and/or risk factors are present (30). IgG2 subclass deficiency is the most common subclass deficiency after IgG4 deficiency and the most common subclass deficiency associated with recurrent infections in children (43). However, the finding of decreased IgG2 in itself is not considered relevant in the presence of a normal humoral immune response to pneumococcal vaccination (43). In IgA deficiency, it may be useful to determine IgG subclasses (especially IgG2 and IgG4), as these often occur together and may be a precursor to CVID (43). Testing of **other IgG subclasses** is rarely indicated, as isolated IgG4 deficiencies are mostly asymptomatic, patients with IgG1 subclass deficiency generally have overall hypogammaglobulinemia and IgG3 deficiencies are generally associated with deficiencies of other subclasses (43). Subclass testing is advised by some authors in cases where despite normal immunoglobulins, infectious problems persist without alternative diagnosis, and when IgA deficiency is diagnosed (43).

- **Complement factors**: when CH50 is very low to undetectable, the CH50 assay should be repeated (Uptodate). When this is repeatedly low to undetectable, the various complement factors and alternative pathway can be assessed. When antibody deficiency is suspected, the most likely factors involved are C1-C4, of which C2 is by far the most common deficiency. Therefore, it is advised to test this first before going on to the other factors. When recurrent *Neisseria* infections are present, both the CH50 and the AP50 assay should be ordered; depending on the result of both assays, testing of individual complement factors can be considered.

- **Additional flow cytometry for lymphocyte typing** (30,44) :
    - HLA-DR typing: activated T-cell lymphocytes
    - CD3+/TCR α/β and γ/δ: subsets of T-cells
    - Memory B-cells
    - Naïve T-cells: CD27, CD45RA cytometry
    - CD40/CD40L: activated T-cells (decreased in HyperIgM)

- **Mitogen stimulation test**: T-cell function can be assessed by mitogen testing (stimulation of T-cells with plant mitogens such as phytohaemagglutinin (PHA), pokeweed mitogen (PWM), Concanavalin A (ConA) or interleukin-2 + CD3 (44). In these assays, T-cells are incubated with mitogens that aspecifically activate the T-cells. The resulting activation can be measured in numerous ways (44), but usually involves quantification of radionuclide-labeled nucleosides which are incorporated in the cells at the end of the incubation period (44). This way, dividing cells incorporating the nucleosides into their DNA can be quantified. Flow cytometry methods are currently also available to measure T-cell response by detecting activation markers. In SCID, the response to mitogens is typically < 10% of the normal response; some forms of "leaky" SCID will show some response, but typically < 30% (44).

- **Antigen stimulation test**: Assessment of specific T-cell function by stimulation with antigens such as tetanus, mumps, Candida, which the patient has most likely been in contact with throughout his/her lifetime (the test may therefore be less useful in very small children) (44). These assays require longer incubation of lymphocytes compared to mitogens (6-7 days versus 72 hours) and response is less strong than with mitogens, due to the polyclonal nature of the mitogen response.

- **Interleukin-12 receptor β1 chain and interferon-γ receptor 1** deficiencies can cause "Mendelian susceptibility to mycobacterial diseases", by disturbing communication between macrophages and T-cells (deficient interferon-γ secretion and signaling, respectively).

- **STAT1 phosphorylation**: both STAT-1 loss of function mutations and gain of function mutations lead to immunodeficiencies. Loss of function mutations, leading to STAT-1 deficiency, cause MSMD, while hyperphosphorylation of STAT-1 (gain of function) predisposes for mucocutaneous candidiasis.

- **Toll-like receptor testing**: the Toll-like receptors (TLR) are part of the innate immune system and therefore usually lead to symptoms early in life (< 2 years) (45). Deficiencies in TLR are rare but should be considered when serious infectious problems persist and other causes are excluded. A large volume (10 ml of blood) is needed for these tests. The TLR are stimulated in a mononuclear cell culture and reaction of the white blood cells, in terms of production of interleukins and TNF-α, is detected.

- **Glucose-6-phosphate deficiency**: severe G6PD can lead to recurrent infections, clinically indistinguishable from chronic granulomatous disease, in addition to the better-known haemolytic anaemia. In cases presenting with chronic granulomatous disease in combination with haemolytic crises and a normal oxidative burst assay, G6PD should be excluded.

Third line tests should always be discussed with the Immunology department of the referral hospital, as indication and interpretation are often not straightforward.

Fourth line tests

The eventual end-point of the protocols, the diagnosis of a PID, is usually obtained by targeted mutation analysis (with the exception of CVID, for which no diagnostic mutations have been described). These tests are not included in the protocols as they depend on the findings of earlier tests. Many possible mutations have been described, therefore mutation analysis is usually performed in specialized Immunology centres after genetic counselling (20).

Protocol 1: recurrent ENT and airway infections, recurrent invasive infections with encapsulated organisms

		Test	Criteria for second/third line test
E	□	WBC count + differentiation	
E	□	Hemoglobin	
E	□	RBC count	
E	□	Platelet count	
S	□	IgG, IgA, IgM	
S	□	Vitamin B12	Hypogammaglobulinemia
S	□	Folic acid	Hypogammaglobulinemia
U	□	Urine sediment	Hypogammaglobulinemia (to exclude urinary loss of immunoglobulins)
S	□	Pneumokokken polysaccharide AS	- hypogammaglobulinemia - normal WBC and immunoglobulins but persisting infections and/or presence of risk factors (bronchiectasis, family history, at least 1 pneumonia)
S	□	CH50	- normal WBC and immunoglobulins but persisting infections and/or presence of risk factors (bronchiectasis, family history, at least 1 pneumonia) - SLE at young age
E	□	T/B/NK flow cytometry	Hypo- or agammaglobulinemia
S	□	IgG2/IgG3/IgG4	- IgA deficiency - normal immunoglobulins and normal humoral vaccine response but persisting infections and/or presence of risk factors (bronchiectasis, family history, at least 1 pneumonia)
H	□	Mitogen testing (PHA, PWM, ConA, IL-2 + anti-CD3)	Consider in case of agammaglobulinemia or lymphopenia
E	□	Memory B-cells	- hypo- and agammaglobulinemia - IgM increased or normal but other immunoglobulins decreased
E	□	CD40/CD40L	IgM increased or normal; other immunoglobulins decreased
S	□	C2	CH50 repeatedly very low (< 20%) to undetectable
S	□	C3/C4	CH50 repeatedly very low to undetectable and C2 normal
S	□	C1q	CH50 repeatedly very low to undetectable and C2 normal
S	□	AP50	- CH50 repeatedly very low (< 20%) to undetectable & C2 normal - recurrent Neisseria infections

H □ Toll-like receptor testing	- recurrent infections with encapsulated bacteria - onset before the age of 2 years

Protocol 2: failure to thrive, unusual infections/unusually severe course, recurrent yeast/fungal infections

First line tests			Criteria for second/third line test
E	□	WBC count + differentiation E □ Haemoglobin E □ RBC count E □ Platelet count	
E	□	T/B/NK flow cytometry	
S	□	IgG, IgA, IgM	
S	□	IgE	
E	□	CFTR mutation	
S	□	tTG-IgA (coeliac disease)	
E	□	HIV Ag/Ab	
S	□	Gliadin IgG	Children < 2 years or IgA deficiency (to exclude coeliac disease)
S	□	Vitamin B12	Hypogammaglobulinemia
S	□	Folic acid	Hypogammaglobulinemia
U	□	urine sediment	Hypogammaglobulinemia (to exclude urinary loss of immunoglobulins)
S	□	Pneumokokken polysaccharide AS	Hypogammaglobulinemia
E	□	Naïve T-cells (CD27, CD45RA)	- agammaglobulinemia or lymphopenia present - gammaglobulins and lymphocytes normal but no improvement, no other diagnosis
E	□	HLA-DR	- agammaglobulinemia or lymphopenia present - gammaglobulins and lymphocytes normal but no improvement, no other diagnosis
E	□	α/β and γ/δ within T-cell populations	- agammaglobulinemia or lymphopenia present - gammaglobulins and lymphocytes normal but no improvement, no other diagnosis
E	□	CD40/CD40L	IgM increased or normal & other immunoglobulins decreased
E	□	Memory B-cells	- hypo- and agammaglobulinemia - IgM increased or normal but other immunoglobulins decreased
H	□	Mitogen testing (PHA, PWM, ConA, IL-2 + anti-CD3)	- agammaglobulinemia or lymphopenia - gammaglobulins and lymphocytes normal but no improvement, no other diagnosis
H	□	Antigen stimulation (Tetanus toxoid, Mumps, Varicella, …)	- agammaglobulinemia or lymphopenia - gammaglobulins and lymphocytes normal but no improvement, no other diagnosis
E	□	IL12-R-β1	Gammaglobulins and lymphocytes normal but no improvement, no other diagnosis
H	□	IFN-γ R1	Gammaglobulins and lymphocytes normal but no improvement, no other diagnosis

H □ STAT1 phosphorylation	Gammaglobulins and lymphocytes normal but no improvement, no other diagnosis

Protocol 3: recurrent pyogenic infections, recurrent yeast/fungal infections

		First line tests (follow up full blood count over time! Do not investigate during an infectious episode)	Criteria for second/third line test
E	□	WBC count + differentiation	
E	□	Peripheral blood smear	
E	□	Haemoglobin	
E	□	RBC count	
E	□	Platelet count	
		Second line tests	
E	□	ESR	Neutropenia
P	□	CRP	Neutropenia
S	□	ANA	Neutropenia
S	□	C3/C4	Neutropenia
S	□	RF (reuma factor)	
S	□	ANCA	Neutropenia
S	□	IgG, IgA, IgM	Neutropenia
S	□	Vitamin B12	Neutropenia & anemia; pancytopenia
S	□	Folic acid	Neutropenia & anemia; pancytopenia
S	□	Copper & ceruloplasmin	Neutropenia
H	□	CD11/CD18	- neutrophilia - delayed umbilical cord separation (> 2 weeks)
H	□	DHR oxidation	Normal neutrophile count
S	□	IgE	Normal neutrophile count
B	□	bone marrow analysis/biopsy with genetic analysis	- neutropenia with other causes excluded - pancytopenia
E	□	T/B/NK flow cytometry	Neutropenia with other causes excluded
H	□	Toll-like receptor testing	- recurrent infections with encapsulated bacteria - onset before the age of 2 years
E	□	G6PD	- features of chronic granulomatous disease but oxidative burst assay normal - hemolytic episodes

Protocol 4: recurrent infections with the same pathogen; unusual infections (also perform protocol 2)

Tests depending on recurrent organism	
	Recurring organism
E □ IL12-R-β1	Atypical mycobacteria and/or Salmonella
H □ IFN-γ R1	Atypical mycobacteria and/or Salmonella
H □ STAT1 phosphorylation	- atypical mycobacteria and/or Salmonella - chronic mucocutaneous candidiasis
S □ AP50	Recurrent Neisseria infections
S □ CH50	Recurrent Neisseria infections
H □ Toll-like receptor testing	HSV encephalitis in child < 6 months
E □ T/B/NK flow cytometry	recurring HSV, EBV, Varicella infections

COMMENTS

TO DO/ACTIONS

1) Discuss the testing strategy with specialists from the referral center
2) Discuss the testing strategy with pediatricians from imelda hospital
3) Implement the testing strategy and evaluate after 1 year of implementation

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ATTACHMENTS

Attachment 1

Summary of PID categories and their most prominent clinical and laboratory features (adapted from Tangye et al, 2020) (2)

Category of PID Relative frequency among PID in %	**Part of immune system involved**	**Relevant subcategories**	**Diagnostic laboratory tests and/or features**	**Clinical presentation**	**Age of presentation**
Antibody deficiencies 45%	Humoral (antibody) response – B-cell maturation	Agammaglobulinemia	All immunoglobulins severely decreased to undetectable	- Recurrent ENT and respiratory tract infections - Recurrent infections with encapsulated bacteria - Persistent/ recurrent *Giardia lamblia* or *Salmonella* infections - CVID: frequently immune dysregulation (auto-immune hematological disorders)	> 6 months – mean age of diagnosis +/- 5 years
		Common variable immune deficiency 1/25.000 individuals	IgG decreased AND IgA *or* IgM decreased AND decreased vaccine response		Adolescence to adulthood
		Hyper IgM (Class switch recombination defects)	IgG and IgA decreased to absent, IgM increased T-cells and/or neutrophils are also often affected		> 6 months of age
		IgG subclass deficiency	IgG subclass decreased – most commonly IgG2 Normal IgG	Variable presentation – usually asymptomatic but may be associated with recurrent infections (cfr above) Present in a sizeable proportion of the normal population	2 – 6 years
		Selective IgA deficiency	IgA undetectable IgG and IgM normal		> 4 years Often diagnosed in adulthood
		Specific antibody deficiency	Decreased vaccine antibody response	Recurrent, predominantly respiratory tract bacterial infections	> 2 years; difficult to diagnose before this age
		Transient hypogammaglobulinemia of infancy (THI)	Immunoglobulins decreased (mostly IgG and IgA)		6 months – 3 years
Combined cellular & humoral immunodeficiencies (CID) 6%	Both cellular (T-cells) and humoral (B-cells) components are affected	Severe Combined Immunodeficiency (SCID) - T-B- - T-B+ 1/58.000 births	T- and B-cells decreased to absent; immunoglobulins decreased to absent Neonatal screening for SCID (TREC) is done in some countries but not Belgium	- Unusual infections (fungal, Pneumocystis, viral, bacterial) - Failure to thrive - Chronic/persistent diarrhea - Recurrent yeast, fungal or viral infections	3 months – 2 years
		Less profound CID	T- and B-cells may be decreased but are sometimes normal; vaccine response and T-cell mitogen response decreased		2 – 6 years of age
CID with syndromic features 13%	Variable	Many subcategories based on primary defect	Variable	Syndromic presentation; many organs involved	From birth Some conditions only become apparent

					during childhood (*e.g.* ataxia-telangiectasia)
Phagocyte defects 5%	Neutrophiles & macrophages	Quantitative defects: congenital neutropenia/pancytopenia	Neutropenia	Pyogenic infections, granulomas, poor wound healing, periodontitis, severe stomatitis, delayed umbilical cord detachment	0 – 6 months
		Qualitative defects: - Motility : Leukocyte Adhesion deficiency (LAD) - Respiratory burst: Chronic granulomatous disease (CGD)	Often neutrophilia CD11/CD18 flow cytometry (LAD1) CD15a flow cytometry (LAD2) 123-DHR flow cytometry (CGD)		
Complement deficiencies 5% - very common for lectin pathway deficiencies	C1-C2-C4 deficiencies	Defective activation classical pathway	Absent CH50 activity	Systemic lupus erythematosus (SLE), infections with encapsulated organisms	Childhood onset of SLE Median age of diagnosis > 18 years
	C3 deficiency	Defective opsonization, defective humoral immune response	Absent CH50 and AH50 activity	Bacterial infections; membranous glomerulonephritis	< 1 year of age
	C5-C9 deficiency	Defective bactericidal activity	Absent CH50 and AH50 activity	Systemic and/or recurrent *Neisseria* infections (often involving rare serotypes)	Adolescence (median age of onset 14-17 years)
	Factor B, factor D, Properdin deficiency	Defective bactericidal activity	Absent AH50 activity		
	Factor I, Factor H deficiencies	Spontaneous activation alternative complement pathway	Variable CH50/AH50, mutation analysis	Atypical HUS, preeclampsia, *Neisseria* infections	Often > 18 years of age (*e.g.* during pregnancy)
	Lectin pathway impairment	Defective bactericidal activity (in theory)	MBL deficiency Collectin polymorphisms Ficolin polymorphisms MASP2 deficiency	Often not associated with a clear clinical phenotype Deficiencies in lectin pathway do not decrease life span!	Increased risk of infection usually seen in infants or otherwise immunosuppressed children (chemotherapy)
Innate immune defects 2%	Interferon-γ pathways IL-12, IL-23 pathways STAT-1 deficiency	Mendelian susceptibility to mycobacterial disease (MSMD)	IL-12 receptor B1 flow cytometry Interferon-γ receptor 1 flow cytometry Mutation analysis	(Recurrent) mycobacterial infection *Salmonella* infections	< 3 years of age; rarely in adolescence and adulthood (46)
		Predisposition to severe viral infection (including attenuated viral vaccines)		Severe viral infections, mycobacterial infections, vaccine-strain infections	
	Toll-like receptor 3 IFN-α, -β, -γ	Herpes simplex encephalitis	Toll-like receptor function analysis Mutation analysis	Herpes simplex encephalitis	0 – 6 months

	STAT-1 hyperphosphorylation (gain-of-function) IL-17 pathways	Predisposition to mucocutaneous candidiasis	STAT1 phosphorylation analysis Mutation analysis	Chronic mucocutaneous candidiasis HSV infection Auto-immunity	2 – 6 years
	TLR signaling pathway deficiencies IL-1 pathways	Pyogenic bacterial infections	Toll-like receptor function analysis Mutation analysis	Bacterial infections Staphylococcal infections	0 – 24 months (45)
Immune dysregulation 3%	Cellular immunity	Familial hemophagocytic lymphohistiocytosis (HLH)	NK cells and CD8+ T-cells activity decreased to absent	Fever, HLH, cytopenias	Often < 18 months, but can present at any age
		Regulatory T-cell defects	CD4+ T-cells decreased or function decreased B cells may be low or normal	Lymphoproliferation, auto-immunity, recurrent viral infections (EBV, CMV)	Early childhood
		PID with prominent auto-immunity	Decreased to normal T- and B-cells Mutation analysis	Auto-immunity, variable lymphoproliferation, eosinophilia, viral infections	Early childhood
		Lymphoproliferative conditions (with/without susceptibility EBV)	Variable – often normal to increased T-cells, hypogammaglobulinemia Auto-immune anemia, thrombopenia	Lymphoproliferation Serious EBV infections Auto-immunity (HLH, IBD)	Early childhood
Auto-inflammatory 6%	Innate immunity T-cells	Type 1 interferonopathies	Mutation analysis T- and B-cell patterns not assessed	Recurrent fevers Auto-immunity	
		Inflammasome defects			
		Non-inflammasome related conditions			

Attachment 2

Scoring System with Clinical and Laboratory Tests for Individuals in Kindreds with HIES

	Points[a]									
Clinical Findings	0	1	2	3	4	5	6	7	8	10
Highest serum-IgE level (IU/ml)[b]	<200	200–500			501–1,000				1,001–2,000	>2,000
Skin abscesses	None		1–2		3–4				>4	
Pneumonia (episodes over lifetime)	None		1		2		3		>3	
Parenchymal lung anomalies	Absent						Bronchiectasis		Pneumatocele	
Retained primary teeth	None	1	2		3				>3	
Scoliosis, maximum curvature	<10°		10–14°		15°–20°				>20°	
Fractures with minor trauma	None				1-2				>2	
Highest eosinophil count (cells/μl)[c]	<700			700–800			>800			
Characteristic face	Absent		Mildly present			Present				
Midline anomaly[d]	Absent					Present				
Newborn rash	Absent				Present					
Eczema (worst stage)	Absent	Mild	Moderate		Severe					
Upper respiratory infections per year	1-2	3	4–6		>6					
Candidiasis	None	Oral	Fingernails		Systemic					
Other serious infections	None				Severe					
Fatal infection	Absent				Present					
Hyperextensibility	Absent				Present					
Lymphoma	Absent				Present					
Increased nasal width[e]	<1 SD	1–2 SD		>2 SD						
High palate	Absent		Present							
Young-age correction	>5 years			2–5 years		1–2 years		≤1 year		

[a] The entry in the furthest-right column is assigned the maximum points allowed for each finding.
[b] Normal <130 IU/ml.
[c] 700/μl = 1SD, 800/μl = 2 SD above the mean value for normal individuals.
[d] For example, cleft palate, cleft tongue, hemivertebrae, other vertebral anomaly, etc. (see Grimbacher et al. 1999*a*).
[e] Compared with age- and sex-matched controls (see Farkas et al. 1994).

Source: Grimbacher et al, 1999 (36)