AJMHS Vol. 47 (2/3): 1-17 © 2016

Establishing a Primary Immunodeficiency Diagnosis: Increasing the Awareness about a Not So Uncommon Pediatric Condition

Genc Sulcebe

Laboratory of Immunology and Histocompatibility, Department of Laboratory Medicine, University of Medicine, Tirana and University Hospital Center "Mother Teresa", Tirana, Albania.

Abstract

Background: Primary immunodeficiencies (PID) include a large group of disorders of the immune system that comprise nearly 300 genetic errors of this system. They include a multitude of clinical presentations ranging from the benign asymptomatic immunoglobulin A deficiency to potentially life-threatening diagnoses, such as severe combined immunodeficiency (SCID). PIDs are characterized by an increased susceptibility to infections as well as a predisposition for malignant and autoimmune manifestations due to a dysregulation of the immune system.

Epidemiology and classification: The prevalence of PID in Europe seems to be at least 6 in 100000 inhabitants, although the data provided by different countries vary enormously. They depend strongly on the local capabilities to achieve or not an exact diagnosis, the correct organization of a reporting and registry system, the geographic region and also from the population or ethnicity characteristics. A screening SCID genetic test on newborns has recently been implemented in the USA helping to provide an early diagnosis, which is essential for a successful early therapeutic intervention.

However, there is a general consensus that the number of diagnosed and reported PID is by far lower that their real number. The International Union of Immunological Societies (IUIS) PID expert committee has proposed a detailed PID classification, which is updated every two years in order to include recent information. European Society for Immunodeficiencies (ESID) has also compiled detailed recommendations for PID diagnosis and registry organization. Based on their principal mechanisms, PID is actually grouped into nine disease categories and the assignment of a clinical phenotype to a precise PID diagnosis requires specialized pediatric care.

Conclusions: Patients with a PID may first be presented to a general pediatrician or a generalist, but also many other medical disciplines can encounter this group of patients. Therefore, all physicians handling with general population healthcare and who may lack familiarity with PID need to be aware of this group of diseases in order to use some easy-to-follow algorithms in order to establish a preliminary PID diagnosis and to properly refer these patients to a more specialized health care level. The final correct diagnosis must be provided by a skilled team including at least an immunologist, an infectious disease specialist and also the referring clinician.

Keywords: Primary immunodeficiencies, severe combined immunodeficiency, common variable immunodeficiency, Albanian Population, prevalence of primary immunodeficiencies

Address for correspondence: Genc Sulcebe, Laboratory of Immunology and Histocompatibility, Department of Laboratory Medicine, University of Medicine, Tirana and University Hospital Center "Mother Teresa", Tirana, Albania. E-mail: genc.sulcebe@umed.edu.al Phone 0692098648

DEFINITION

Primary immunodeficiencies (PID) include a large group of genetic disorders resulting from one or more abnormalities of the immune function and/or regulation (1). Since the first description of the primary agammaglobulinemia in 1952, the number of genetically-defined inborn errors of immunity has increased enormously with nearly 300 gene defects contributing to a multitude of clinical presentations ranging from the asymptomatic IgA deficiency to life-threatening diagnoses, such as severe combined immunodeficiency's (SCID) (2). PID are characterized not only by an increased susceptibility to infections but also by a dysregulation of the immune system functions that may predispose to malignancy, autoimmune diseases, allergy and auto-inflammation (3). PID have served as "experiments of nature in humans" and their study has made possible the detailed knowledge of genes and mechanisms involved in the biology of the immune system.

In many cases, T-cell PID are also associated with B-cell deficiencies in the clinical form of severe combined immunodeficiency (SCID). The patients with SCID express their clinical signs within the first months of life. SCID is characterized by a complete block of T-cell development but also Natural Killer (NK) and B cell number and/or function can be severely affected. Combined immunodeficiency syndromes (CID) have a less severe clinical picture and they are caused mostly by hypomorphic mutations in SCID linked genes or from partial abnormalities of T-cell development. The patients affected by CID express their clinical signs later in childhood by a high frequency of recurrent infections that are often accompanied with a deregulation of the immune system in the form of autoimmunity and/or abnormal lymphoproliferation (4,5).

Over the last few years, impressive progress has been made not only in understanding the disease mechanisms of PID but also in improving the long-term outcomes of potentially curative treatments, including hematopoietic stem cell transplantation (HSCT) and gene therapy (6,7, 8,9). A screening SCID genetic test on newborns has recently been implemented in the USA helping to provide an early diagnosis, which is essential for a successful therapeutic intervention in these patients (10).

PID are indeed more common than generally realized and it is increasingly becoming evident that PID recognized till now constitute only the visible part of a large "iceberg". One factor contributing to the known significant low detection rate of a PID is the low index of suspicion for these diseases, which often derives from a low awareness about this disease group. Another factor influencing the low PID detection rate and also the delay of a PID diagnosis is the lack of availability to carry out systematically all the detailed immunological and genetic laboratory examinations needed to detect the large range of abnormalities affecting the different facets of the immune system. The delay to a PID diagnosis is also related to the

PID type. It is known that common variable immune deficiency (CVID), one of the most frequent PIDs has an estimated delay of 6–8 years in diagnosis after the onset of symptoms and this delay can be more than 3 years for other hypogammaglobulinemias (11).

The rarity of many forms of PID requires national coordination and international collaboration in order to drive improvements in detection, management, and therapeutic strategies and also to facilitate translational research in this field. PID management is resource-intensive, and therefore epidemiological data of high quality are of paramount importance in making health-care planning decisions in this field (12,13).

Epidemiology

Epidemiological data on PID are difficult to obtain and the reports depend strongly on the local capabilities to achieve or not an exact diagnosis, the correct organization of a reporting and registry system, the geographic region, the different methodologies used to register PID and also from the population characteristics, primarily related to the rate of consanguineous marriages in the specific population. Therefore, incidence and prevalence data vary enormously in different reports. For example, in a paper about the PID epidemiology in the UK, Edgar et al report that the incidence of PIDs requiring treatment ranges from 1:20 000 to 1:500 000 depending on the exact diagnosis and the studied population (1). As following the data released by the European Society for Immunodeficiencies (ESID), the minimal incidence in Europe might be around 1:3000 to 1:4000 per year and the prevalence at least 6 for 100,000 inhabitants (13), although the data provided by well documented national studies (14,15,16) and also by ESID Registry (17) show high variability most likely related to deficiencies of data entry in several countries (13). The PID prevalence rates reported in Europe in 2014 by ESID (17) are shown in **Table 1**. They are extremely various and range from 0.056 (Romania) to 6.164 (France) per 100 000 inhabitants. However, there is a general consensus that the number of diagnosed and reported PIDs is far lower that their real number (11,13).

Referring to USA data, the prevalence of any PID diagnosis ranged from 41.1 to 50.5 per 100,000 in the year 2007. The prevalence was two-fold higher among Whites as among Blacks or Hispanics. This study was conducted using a cross-sectional survey in order to estimate the prevalence of PID using related ICD-9 codes (18).

The prevalence rates are different for individual PID, ranging from the most common and often asymptomatic IgA deficiency (1:233 to 1:3000) to the very serious forms of SCID (1:58000) (19). As far as concerns PID requiring treatment, humoral immunodeficiencies are the most frequently encountered and among them common variable immunodeficiency (CVID) has the highest prevalence.

Countries	Prevalence (cases per 100 000 inhabitants)
France	6.164
Spain	4.947
Switzerland	4.182
Netherlands	4.047
Hungary	3.765
United Kingdom	3.705
Estonia	3.059
Turkey	2.355
Czech Republic	2.317
Germany	2.144
Belgium	2.143
Ireland	1.987
Italy	1.955
Greece	1.739
Poland	1.44
Austria	1.178
Slovenia	1.049
Sweden	1.02
Portugal	0.714
Serbia	0.627
Romania	0.056

Table 1. Minimal prevalence rates of PIDs in European countries as reported by European Society for Immunodeficiencies (ESID) (living cases per 100 000 inhabitants in the year 2014) shown in a decreasing order (Reference 18: http://esid.org/Working-Parties/Registry/ESID-Database-Statistics).

Less frequently reported PID categories are predominant T-cell disorders, granulocyte disorders, and the "other well-defined immunodeficiency" syndromes (3, 18,20).

As following the 2014 ESID Registry data, the predominantly antibody disorders are the most frequent PIDs observed (55.66%), followed by "other well defined PIDs" (13.91%), phagocyte disorders (8.73%), predominantly T-cell deficiencies (7.47%), autoimmune & immune dysregulation syndromes (3.89%), auto-inflammatory syndromes (2.06%), unclassified PIDs (1.4%) and defects in innate immunity (1%) (13).

Predominantly antibody disorders are more prevalent in adults than in children and within this category, CVID is the most frequent PID encountered.

Chronic granulomatous disease (CGD) is the most prevalent PID among phagocyte disorders.

In a national study conducted in Tunisia, the estimated prevalence rate was 4.3 per 100,000 inhabitants (21). The prevalence of different PIDs according to the International Union of Immunological Societies (IUIS) classification, was as follows: combined T-cell and B-cell immunodeficiency disorders were the most frequently found (28.6%), followed by

phagocytes abnormalities (25.4%), other well-defined immunodeficiency syndromes (22.7%), predominantly antibody deficiency diseases (17.7 %), diseases of immune dysregulation (4.8%), defects of innate immunity (0.4 %) and complement deficiencies (0.4%). Recurrent infections, particularly lower airway infections (62.3 %), were the most common manifestations in these PID patients.

Diagnostic criteria: How to reach a PID diagnosis?

Suspicion of immunodeficiency is raised when recurrent infections occur in multiple locations and these infections are unusually severe, complicated, and resistant to antibiotic treatment. The infections are characterized by their length and severity and often they are caused by unusual organisms such as *Pneumocystis jiroveci*, *Giardia lamblia* or atypical mycobacteria. The characteristic clinical presentation of PID involves frequent and often severe pulmonary tract infections (recurrent bronchitis and/or pneumonia). Other frequently described symptoms of a PID are intestinal infections. Chronic diarrhea, especially when it is caused by unusual bacteria (e.g., Yersinia or Campylobacter), fungi (e.g., Cryptosporidium), or even common infections such as persistent rotavirus or Salmonella, accompanied by the presence of failure to thrive should trigger an immunologic workup for PID in infants or young children (11).

Other clinical signs include skin lesions (e.g., eczema, warts, abscesses, pyoderma, and alopecia), oral or esophageal thrush, oral ulcers, and periodontitis. In addition to the risk of infection, PID patients have an increased risk for autoimmune diseases, like immune thrombocytopenic purpura (ITP), presumably related to the immune dysregulation. Significantly, there are often delays of many years in the diagnosis of some PIDs, and a late diagnosis increases the risk of chronic organ damage such for example the reported correlation between the delayed diagnosis of X-linked agammaglobulinemia (M. Bruton) and development of bronchiectasis (22).

Recurrent and severe infections of the respiratory tract with encapsulated bacteria like *Streptococcus pneumoniae* and *Haemophilus influenzae* or gastrointestinal infection with *Giardia lamblia* and *Campylobacter jejuni* evoke the presence of an antibody deficiency, whereas recurrent or severe Candida infection, Pneumocystis jiroveci pneumonia and severe viral infections such as cytomegalovirus (CMV), human papilloma virus (HPV) etc. are clinical signs of a T cell deficiency. Recurrent *Neisseria meningitidis* infections are very evocative signs of deficiencies of the common terminal complement components (11).

In order to recognize the children affected by a PID, the clinicians must be very careful to identify the evocative signs as early as possible. In a campaign aiming to educate both families and clinicians, a group of 10 warning signs for PID has been promoted by the Jeffrey Modell Foundation Medical Advisory Board (11). These

warning signs are described in **Table 2**. The difficult issue for the pediatrician is that many normal children can have one or more of these 10 potential warning signs. Therefore, detecting children with a potential PID will require not only a high index of suspicion but also a good clinical experience in order to properly identify whether a child's global clinical presentation is out of the ordinary, compared with other children encountered in the common practice.

A group of investigators has studied the above mentioned 10 warning signs and have concluded that these clinical signs were not developed using evidence-based studies in order to determine their predictive ability (23). They reviewed the records of 430 children who had a definitive PID diagnosis in two immunology clinics in Northern England over a 10-year period. A comparison group included 133 children who presented to these centers with concerns of PID but who did not have PID after evaluation. From the 10 warning signs, they concluded that only 3 of them were significantly predictive of a child having a definable PID: 1 - A positive family history of PID; 2 - Requiring ≥ 2 months of antibiotics without improvement; 3 - Failure to thrive.

Pediatric patients

1. Four or more new ear infections within 1 year.
2. Two or more serious sinus infections within 1 year.
3. Two or more months on antibiotics with little effect.
4. Two or more pneumonias within 1 year.
5. Failure of an infant to gain weight or grow normally.
6. Recurrent, deep skin or organ abscesses.
7. Persistent thrush in mouth or fungal infection on skin.
8. Need for intravenous antibiotics to clear infections.
9. Two or more deep-seated infections including septicemia.
10. A family history of Primary immunodeficiency.

Adult patients

1. Two or more new ear infections within 1 year.
2. Two or more new sinus infections within 1 year, in the absence of allergy.
3. One pneumonia per year for more than 1 year.
4. Chronic diarrhea with weight loss.
5. Recurrent viral infections (colds, herpes, warts, condyloma).
6. Recurrent need for intravenous antibiotics to clear infections.
7. Recurrent, deep abscesses of the skin or internal organs.
8. Persistent thrush or fungal infection on skin or elsewhere.
9. Infection with normally harmless tuberculosis-like bacteria.
10. A family history of Primary immunodeficiency.

Table 2. Ten warning clinical signs for PIDs developed by the Jeffrey Modell Foundation Medical Advisory Board (Reference 11).

No differences in the number of deep-seated infections or episodes of pneumonia or abscesses were found between the children with and without definable PID. Paradoxically, a history of frequent episodes of acute otitis media or sinus infections was associated with a lower risk of definable PID. The conclusion of these investigators is that a PID information campaign must be directed to the hospital pediatricians and also families with a history of PID rather than the general public (23).

LABORATORY EXAMINATIONS

Humoral Immunity

Quantitative and qualitative testing of serum immunoglobulins and specific antibodies can reveal low levels of IgG and/or IgA and IgM, which may be primary or secondary. The significance of a selective low IgM is unclear although it can be associated with aging, autoimmunity, and lymphoproliferative disease. A selective low IgA is found in many apparently healthy individuals, although it may be eventually associated with autoimmunity, allergy and an increased frequency of infections. A low IgG level may represent significant immunodeficiency, especially when associated with a history of unusual or recurrent respiratory or gastrointestinal infections. Very low total serum IgE levels (for example <2 international units/ml) may also represent antibody deficiency. Examination of these serum samples by testing of other immunoglobulin isotypes can lead to a diagnosis of antibody deficiency. Reference ranges of immunoglobulin levels are age-dependent, therefore they levels should be considered carefully in function of age-related normal ranges (24).

Even when a normal level of serum immunoglobulins or a normal B lymphocyte number is encountered, a PID diagnosis implicating a defect in antibody production cannot be excluded. A functional test of antibody production capacity is needed in these cases. At the best, this is carried out by measuring the increase in antibody levels after immunization with harmless vaccines and antigens such as tetanus and/or diphtheria toxoids as well as pneumococcus polysaccharides. A significant increase in these levels 4 weeks after a booster immunization testifies a normal antibody-producing function of B-cells even in the case of the presence of abnormal serum immunoglobulin levels (24). Also, a negative EBV antibody result especially in adults, or an absent or very low antistreptolysin O antibody level even after streptococcus infections should raise the suspicion of an underlying PID.

Patients with PIDs respond poorly to routine vaccinations. Failed vaccine responses, especially when some of them are implicated, are suggestive of an antibody immune-deficiency. When these vaccines contain live organisms, they carry a significant risk of disseminate and severe infection for PID patients especially for those with SCID (25).

The sera of normal individuals contain "natural" anti-A and anti-B blood group antibodies of the IgM isotype, also called iso-hemagglutinins, in correlation to the cell group (eg, a patient with blood group A will have anti-B antibodies, but no anti-A antibodies). The young infants may not have natural anti-A and -B antibodies until approximately 3 months of age, but in other ages, a lack of these antibodies may indicate the presence of an immunodeficiency (24).

Lymphocyte count and function

Persistent unexplained lymphopenia may suggest the presence of a PID particularly in the first few months of life. A low lymphocyte count may be a primary or secondary phenomenon (HIV infection for example) and should prompt further investigation of the lymphocyte subsets and also of serum immunoglobulins in order to exclude a SCID (especially in young children).

Aspects of the immune system (IS) to be examined	Laboratory examinations to be carried out
Counting of cells involved in the IS function (T cell subsets, B cells, NK cells, neutrophils, monocytes, eosinophils)	- Complete blood cell count with manual differential - Blood leucocyte immunophenotyping (IFT) by flow cytometry
Examination of T cell function	- *In vivo:* Intradermal skin test with mitogens (Phytohemagglutinin-PHA) and antigens (Candidin, tetanus toxoid) - *In vitro:* Measurement of lymphocyte stimulation and prolifereation by mitogen (PHA), and antigen (candidin and tetanus toxoid) activation in vitro
Examination of B cell function	- Measurement of serum IgG, IgA, IgM, and IgE levels - Measurement of serum antibody levels to specific antigens before and after vaccination against diphtheria, tetanus, and pneumococcus
Examination of complement system function	- Total hemolytic complement assay (CH50), serum C3, C4 and C1-INH testing
Examination of phagocyte function	- Oxidative burst testing with Nitroblue tetrazolium test (NBT) by microscopy or by Dihydrorhodamine (DHR) test through flow cytometry

Table 3. Initial laboratory workup to be carried out in case of consistent suspicion for a PID diagnosis.

SCID is often associated with a pan-lymphopenia, but selective deficiencies in one or other T-cell subpopulations (e.g., selective CD4 T cell deficiency) can be masked within a normal total lymphocyte count.

The number of lymphocytes in infants and young children are significantly higher than in adults. Therefore, their total levels and also the lymphocyte subpopulations must be considered carefully in relation to the age-appropriate normal ranges (26). However, many PID clinical phenotypes may be accompanied with lymphocyte T subsets within the normal age ranges. In these cases, functional in vivo or in vitro T cell examination by mitogen and antigen stimulation must be performed in order to exclude a T cell deficiency **(Table 3)**.

Phagocyte and monocyte count and function

Although inborn low numbers of polymorphonuclear neutrophils are very rare, abnormal phagocyte functions are not so rare and must be included in the immunological workup of PID **(Table 3).**

Monocytopenia has recently been recognized within a new PID caused by GATA2 deficiency causing a predisposition for atypical mycobacteria and human papillomavirus infections and also a high risk for myelodysplasia and acute myeloid leukemia. Although this condition is extremely rare, a persistently absent or very low monocyte count should be considered carefully.

Testing for complement components such as C3, C4, C1INH or total complement hemolytic assay must also be included in the general immunological workup **(Table 3).**

Platelet count

Idiopathic thrombocytopenic purpura (ITP) can be a presenting sign of primary or secondary immunodeficiency (24). Platelet volume is measured in the normal processing of a full blood count but is usually not reported or considered. A low platelet volume is suggestive of Wiskott–Aldrich syndrome. Although this syndrome is very rare, the finding of a low platelet volume should prompt consideration of this diagnosis. Similarly, autoimmune hemolytic anemia and autoimmune neutropenia can be presenting clinical signs of immunodeficiency such as in CVID for example.

Histopathology Examination

Several histopathological abnormalities may be found in different PID. Granulomatous elements have been found in the granulomatous variant of CVID mimicking similar findings in conditions such as *Mycobacterium tuberculosis* infection, sarcoidosis or Crohn's disease. In the granulomatous variant of CVID, low levels of serum immunoglobulins are usually found. In contrast, in sarcoidosis or other inflammatory diseases, the serum immunoglobulins are expected to be raised (24).

In many immunodeficiency syndromes, notably those involving B cells (like agamma-globulinemias or in the hyper-IgM syndrome), the active germinal centers with their characteristic light and dark zones may be absent or abnormal. If similar findings are detected in a

lymph node biopsy, a PID workup should be prompted. An absence of plasma cells in biopsies can be found in certain PIDs with low or absent B cells, such as X-linked or autosomal recessive agammaglobulinemias, or in a subset of patients with CVID (24).

The finding of a villous atrophy on small bowel biopsy is normally consistent with a diagnosis of celiac disease. However, villous shortening may be associated with infections such as Giardia lamblia which is a common infection found in antibody deficiency.

Genetics Testing

In infants with failure to thrive or with various syndromes, it is often necessary to investigate for chromosomal abnormalities through cytogenetic examination. This test involves a mitogenic stimulation of lymphocytes in order to visualize the metaphase chromosomes. A failed cytogenetic test may indicate a quantitative or functional T cell deficiency and may raise the suspicion for serious PID such as SCID. A delayed SCID diagnosis may lead to life-threatening opportunistic infections by Epstein–Barr virus (EBV), cytomegalovirus (CMV) etc, or to severe infections from normally harmful live vaccines including measles or Bacillus Calmette–Guerin (BCG) (27). An early SCID diagnosis can prevent these infections and can make possible to proceed to a successful HSCT or gene therapy.

A large range of molecular biology tests is actually carried out in specialized labs in order to detect the abnormal genes involved and to properly and definitively establish a PID diagnosis on a genetic basis (28, 29, 30).

Classification

Since the advent of next-generation genomic sequencing, the number of PID-related genetic disorders is increasing quickly every year. The International Union of Immunological Societies (IUIS) PID expert committee has proposed a detailed PID classification, which is updated every two years in order to include the recent information gathered. The PIDs are actually grouped into nine categories based on the principal mechanism of each disease. For each individual PID entity, the genotype and the immunological and clinical phenotypes are also described (31). However, this classification and the respective tables are becoming rather complicated and difficult to be managed. Consequently, this detailed IUIS PID expert committee catalog offers limited assistance to most physicians working at the bedside. In **table 4** we show a modified IUIS PID expert committee classification in a more succinct and summarized form.

Another IUIS expert group, based on clinical and immunological PID phenotypes, has developed some detailed algorithms for each of the 9 groups of PID, in order to reach the diagnosis of a particular PID. These diagnostic algorithms, that are conceived to be used in a tertiary health care level, allow a more rapid and accurate molecular diagnosis and genetic counseling, making possible a more appropriate treatment of affected patients (2).

PID Pathogenetic Categories	Main clinical phenotype features	Inheritance	Number of genetic based disease diagnoses	Prevalence rate among all PIDs (cases per 100 000 inh.)
1. Combined T and B cell PIDs (Predominantly T cell)			Total: **50**	0.05 - 0.52
	1. T – B+ SCID	XL or AR	8	
	2. T – B- SCID	AR	8	
	3. CID less profound than SCID (can be presented as T+B+ CID)	XL or AR	34	
2. Combined ID with associated or syndromic features Wiskott-Aldrich; Di George; Ataxia-telangiectasia etc.	10 different clinical entities (can be presented as T+B+ CID)	XL or AR	Total: **45**	0.24 - 0.64
3. Predominantly antibody deficiencies (4 groups)			Total: **34**	1.27 - 2.93
	1. Severe reduction of all Ig isotypes and profound decrease or absent B cells	XL or AR or unknown	9	
	2. Severe reduction in at least 2 serum Ig isotypes and presence or low numbers of B cells	AR, AD or variable	12	
	3. Severe reduction in serum IgG and IgA with normal/elevated IgM and normal numbers of B cells	AR	4	
	4. Isotype or light chain deficiencies with generally normal numbers of B cells	AD, AR, or variable	9	
4. Diseases of immune dysregulation			Total: **37**	0.05 - 0.31
	1. Familial hemophagocytic lymphohistiocytosis (FHL) syndromes	XL, AR	10	
	2. T regulatory cells genetic defects	XL, AR, AD	4	
	3. Autoimmunity with or without lymphoproliferation	AR, AD	9	
	4. Immune dysregulation with colitis	AR, AD	4	
	5. Type 1 Interferonopathies	AR, AD	10	
5. Congenital defects of phagocyte number, function, or both			Total: **29**	0.16 - 0.72
	1. Congenital neutropenias	XL, AR, AD	15	
	2. Defects of Motility	XL, AR, AD	9	
	3. Defects of Respiratory Burst	XL, AR, AD	5	

6. Defects in Intrinsic and Innate Immunity			Total: **33**	0.0 - 0.14
	1. Mendelian Susceptibility to Mycobacterial Disease (MSMD)	AR, AD	11	
	2. Epidermodysplasia verruciformis (EVER def; WHIM syndr.)	AR, AD	3	
	3. Predisposition to severe viral infection (STAT, IRF 7, CD16 def.)	AR	4	
	4. Herpes simplex encephalitis (TLR3, TRAF3, TRIF def)	AD, AR	5	
	5. Predisposition to invasive fungal diseases (CARD9 def.)	AR	1	
	6. Chronic mucocutaneous candidiasis (IL17, STAT1, ACT1 def.)	AD, AR	5	
	7. TLR signaling pathway deficiency (IRAK4, MyD88 def) (Bacterial infections)	AR	2	
	8. Isolated congenital asplenia (RPSA mutation)	AD	1	
	9. Trypanosomiasis (APOL-1 mutation)	AD	1	
7. Autoinflammatory disorders			Total: **21**	0.01 - 0.06
	1. Defects affecting the inflammasome (FMF et.)	AD, AR	9	
	2. Non inflammasome-related conditions (TRAPS syndr. etc)	AD, AR	12	
8. Complement deficiencies			Total: **30**	0.02 - 0.33
	1. Integral complement cascade component deficiencies (C1q – C9 def)	AD, AR, XL	19	
	2. Complement Regulatory defects (C1INH etc)	AD, AR, XL	11	
9. Phenocopies of PID			Total: **10**	
	1. Associated with somatic mutations		4	
	2. Associated with autoantibodies		6	
TOTAL			**289**	**2.0 – 6.0**

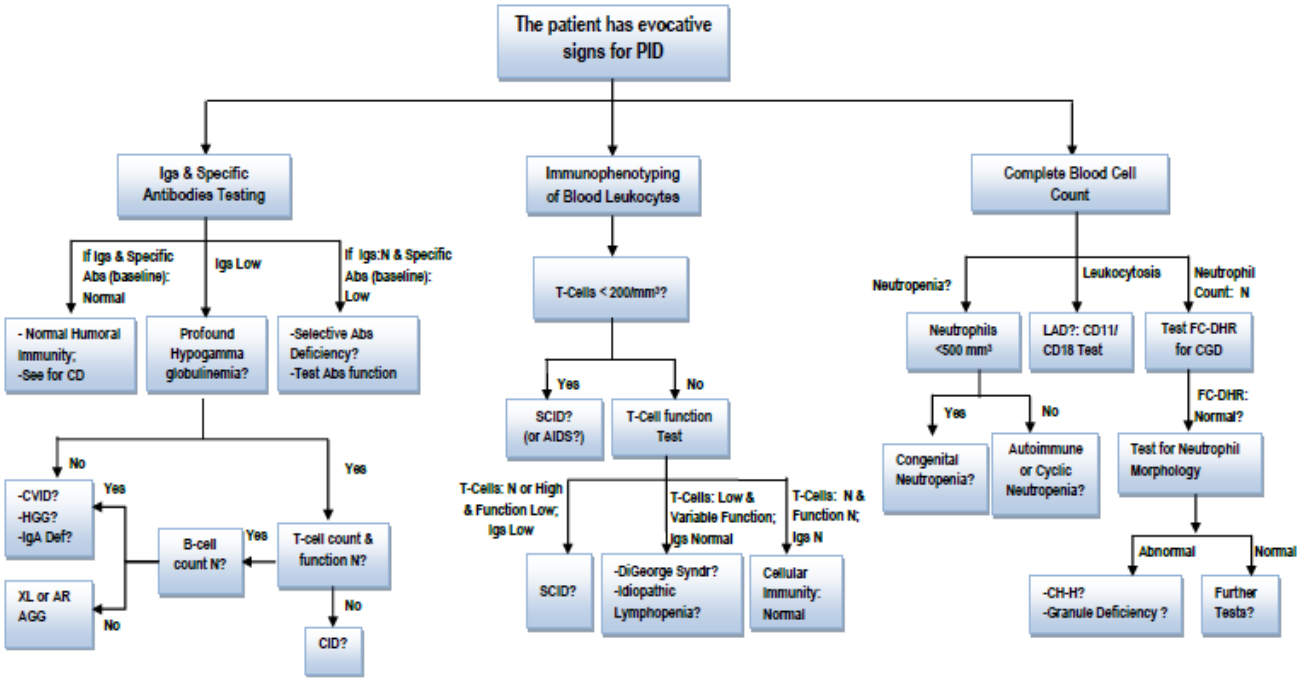
PID – primary immunodeficiency; **SCID** – severe combined immunodeficiency; **CID** – combined immunodeficiency; **XL** – X-linked; **AR** – autosomal recessive; **AD** - autosomal dominant

Table 4. PID IUIS 2015 classification (Ref. 32) modified in a shortened version.

However, patients with PID related clinical signs are usually first presented to a general practitioner or pediatrician not specially trained in PID diagnosis. Especially for those outside the field of PID, those in training or for the general clinicians, a more simplified clinical diagnostic algorithm is needed (32).

Such physicians need an easy-to-follow diagnostic scheme that is based on the clinical and/or biological phenotype that they observe. In order to reach a rapid and preliminary PID diagnosis, a practical scheme has been proposed to be used by the general pediatricians through a relatively simple diagnostic algorithm (11). We have modified this scheme in **Figure 1** in order to condense the diagnostic algorithm in a unique figure.

Figure 1. Schematic presentation of a simplified workup algorithm for primary immunodeficiency diagnostics (modified from Lehman et al; Ref. 11).



Abs – Antibodies; **AIDS** – Acquired Immune Deficiency Syndrome; **AGG** – Agammaglobulinemia; **AR** – Autosomal Recessive; **CD** – Complement Deficiencies; **CGD** – Chronic Granulomatous Disease; **CH-H** – Chediak-Higachi Syndrome; **CID** – Combined Immune Deficiencies; **CVID** – Common Variable Immunedeficiency; **Def.** – Deficiency; **FC-DHR** – Dihydrorhodamine Assay by Flow Cytometry; **Igs** - Serum Immunoglobulins; **HGG** – Hypogammaglobulinemia; **LAD** – Leukocyte Adhesion Deficiency; N- Normal; **PID** – Primary Immunodeficiencies; **SCID** – Severe Combined Immune Deficiencies; **XL** – X-Linked

Data from the Laboratory of Immunology of the University Hospital Center of Tirana

In order to obtain a preliminary estimation of the PID prevalence in Albania, we examined the immunological examination results of 1737 pediatric patients that have been tested in our laboratory for their IgG, A and M serum levels and/or peripheral blood leukocyte subpopulations during a 5 year period (2010-2015). Taking into account that the Laboratory of Immunology of the University Hospital Center of Tirana is the reference immunological laboratory for all Albania, we can consider that most if not all patients with a PID suspicion are sent for examination in this center. From all patients tested during the 5-year time lapse, we detected 40 cases with abnormal serum immunoglobulins and/or lymphocyte subpopulations results accompanied by recurrent infections and without a known primary cause.

Among them, 18 cases were found to be with isolated IgA deficiency, 7 with Bruton agammaglobulinemia (total absence of immunoglobulins and B cells), 4 with isolated IgM, 3 with low IgA and IgM, 3 with low IgG and IgA, 2 cases with low IgG and IgM, 2 cases with isolated IgG and 1 case was diagnosed with SCID. No PID with phagocyte, innate immune system, complement or other dysregulation /autoinflammatory disorders have been detected. The positivity detection rate was 2.3 % and a probably detected prevalence rate can be tentatively proposed at approximately 1.4 cases per 100 000 inhabitants. The real prevalence rate must be indeed higher due to a probable low diagnostic rate of some PIDs in our country. This low detection rate seems to concern mainly the PIDs due to cellular causes (T cell and phagocyte cells) that have a more rapid and severe disease course and that require an early, rapid, and detailed diagnostic workup.

CONCLUSIONS

The consequences of a delayed diagnosis of a PID are recurrent, severe and potentially life-threatening infections and/or chronic organ damage (eg, bronchiectasis). Detailed diagnostic immunologic and genetic examinations must be performed as soon as a PID diagnosis is suspected in these patients. An early and detailed diagnosis will aim to prevent the occurrence of irreversible damages and will make possible appropriate and timely interventions in order to prevent chronic morbidity and mortality (33).

All patients with a PID suspicion must be addressed to a centralized tertiary care pediatric center where they must be submitted to a thorough PID workup based on the clinical algorithms described above. The final correct diagnosis must be provided by a skilled team including at least an immunologist, an infectious disease specialist and also the referring clinician. This center must organize a centralized PID registry conforming to the ESID guidelines (13, 17). Early interventions with regular intravenous immunoglobulins will prevent irreversible complications (20,34,35).

In the case of a cellular PID (abnormal T cell or phagocyte function), the possible interventions (HSCT or gene therapy) are successful if performed within the first months of life (6,7,8). Obligatory newborn BCG vaccination must be critically evaluated in case of families with a PID history because they can be life-threatening in the eventuality of the presence of a T-cell PID.

Acknowledgments: Not available.

Conflict of interest disclosure: Not available.

REFERENCES

1 Edgar JD, Buckland M, Guzman D, Conlon NP, Knerr V, Bangs C et al. The United Kingdom Primary Immune Deficiency (UKPID) Registry: report of the first 4 years' activity 2008-2012. Clin Exp Immunol 2014;175(1):68-78.

2 Bousfiha A, Jeddane L, Al-Herz W, Ailal F, Casanova JL, Chatila T et al. The 2015 IUIS Phenotypic Classification for Primary Immunodeficiencies. J Clin Immunol 2015;35(8):727-38.

3 De Vries E, Driessen G. Educational paper: Primary immunodeficiencies in children: a diagnostic challenge. Eur J Pediatr 2011;170(2):169-77.

4 Pachlopnik Schmid J, Güngör T, Seger R. Modern management of primary T-cell immunodeficiencies. Pediatr Allergy Immunol 2014;25(4):300-13.

5 Eibel H, Salzer U, Warnatz K. Common variable immunodeficiency at the end of a prospering decade: towards novel gene defects and beyond. Curr Opin Allergy Clin Immunol 2010;10(6):526-33.

6 Pai SY, Logan BR, Griffith LM, Buckley RH, Parrott RE, Dvorak CC, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. N Engl J Med 2014;371(5):434-46.

7 Cavazzana M, Touzot F, Moshous D, Neven B, Blanche S, Fischer A. Stem cell transplantation for primary immuno-deficiencies: the European experience. Curr Opin Allergy Clin Immunol 2014;14(6):516-20.

8 Burroughs L, Woolfrey A. Hematopoietic cell transplantation for treatment of primary immune deficiencies. Cell Ther Transplant 2010;2(8). doi: 10.3205/ctt-2010-en-000077.01.

9 Hacein-Bey-Abina S, Pai SY, Gaspar HB, Armant M, Berry CC, Blanche S, Bleesing J et al. A modified γ-retrovirus vector for X-linked severe combined immunodeficiency. N Engl J Med 2014;371(15):1407-17.

10 Buelow BJ, Routes JM, Verbsky JW. Newborn screening for SCID: where are we now? Expert Rev Clin Immunol. 2014;10(12):1649-57.

11 Lehman H, Hernandez-Trujillo V, Ballow M. Diagnosing primary immunodeficiency: a practical approach for the non-immunologist. Curr Med Res Opin 2015;31(4):697-706.

12 Kindle G, Gathmann B, Grimbacher B. The use of databases in primary immuno-deficiencies. Curr Opin Allergy Clin Immunol 2014;14(6):501-8.

13 Mahlaoui N, Gathmann B, Kindle G, Ehl S, on behalf of the ESID Registry Working Party Steering Committee (Isabella Quinti, Italy, Bodo Grimbacher, Germany, Matthew Buckland, United Kingdom, Markus Seidel, Austria, Joris van Montfrans, The Netherlands) and the ESID Society. The European Society for Immunodeficiencies (ESID) Registry: recent advancements in the epidemiology of Primary Immuno-deficiencies and how does that translate in clinical care. Rare Diseases and Orphan Drugs: An International Journal of Public Health 2014, 1(4), Suppl. 4, 25-27.

14 Marschall K, Hoernes M, Bitzenhofer-Grüber M, Jandus P, Duppenthaler A, Wuillemin WA, et al. Swiss PID Registry Working Group. The Swiss National Registry for Primary Immunodeficiencies: report on the first 6 years' activity from 2008 to 2014. Clin Exp Immunol 2015;182(1):45-50.

15 Gathmann B, Goldacker S, Klima M, Belohradsky BH, Notheis G, Ehl S et al. The German national registry for primary immunodeficiencies (PID). Clin Exp Immunol 2013;173(2):372-80.
16 Ludviksson BR, Sigurdardottir ST, Johannsson JH, Haraldsson A, Hardarson TO. Epidemiology of Primary Immuno-deficiency in Iceland. J Clin Immunol 2015;35(1):75-9.
17 European Society for Immunodeficiencies (www.esid.eu.2016).
18 Kobrynski L, Powell RW, Bowen S. Prevalence and morbidity of primary immunodeficiency diseases, United States 2001-2007. J Clin Immunol 2014;34(8):954-61.
19 Kwan A, Abraham RS, Currier R, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA 2014;312:729-38.
20 Ameratunga R, Woon ST, Gillis D, Koopmans W, Steele R. New diagnostic criteria for common variable immune deficiency (CVID), which may assist with decisions to treat with intravenous or subcutaneous immunoglobulin. Clin Exp Immunol 2013;174(2):203-11.
21 Mellouli F, Mustapha IB, Khaled MB, Besbes H, Ouederni M, Mekki N, et al. Report of the Tunisian Registry of Primary Immunodeficiencies: 25-Years of Experience (1988-2012). J Clin Immunol 2015;35(8):745-53.
22 Ebadi M, Aghamohammadi A, Rezaei N. Primary immunodeficiencies: a decade of shifting paradigms, the current status and the emergence of cutting-edge therapies and diagnostics. Expert Rev Clin Immunol 2015;11(1):117-39.
23 Subbarayan A, Colarusso G, Hughes SM, Gennery AR, Slatter M, Cant AJ, Arkwright PD. Clinical features that identify children with primary immunodeficiency diseases. Pediatrics 2011;127(5):810-6.
24 Bright PD, Rooney N, Virgo PF, Lock RJ, Johnston SL, Unsworth DJ. Laboratory clues to immunodeficiency; missed chances for early diagnosis? J Clin Pathol 2015;68(1):1-5.
25 Medical Advisory Committee of the Immune Deficiency Foundation, Shearer WT, Fleisher TA, Buckley RH, Ballas Z, Ballow M, Blaese RM, et al. Recommendations for live viral and bacterial vaccines in immunodeficient patients and their close contacts. J Allergy Clin Immunol 2014;133(4):961-6.
26 Duchamp M, Sterlin D, Diabate A, Uring-Lambert B, Guérin-El Khourouj V, et al. B-cell subpopulations in children: National reference values. Immun Inflamm Dis 2014;2(3):131-40.
27 Mahmoudi S, Khaheshi S, Pourakbari B, Aghamohammadi A, Keshavarz Valian S, Bahador A, et al. Adverse reactions to Mycobacterium bovis bacille Calmette-Guérin vaccination against tuberculosis in Iranian children. Clin Exp Vaccine Res 2015;4(2):195-99
28 Moens LN, Falk-Sörqvist E, Asplund AC, Bernatowska E, Smith CI, Nilsson M. Diagnostics of primary immunodeficiency diseases: a sequencing capture approach. PLoS One 2014;9(12) DOI:10.1371 /journal.pone.0114901.
29 Roos D, de Boer M. Molecular diagnosis of chronic granulomatous disease. Clin Exp Immunol 2014;175(2):139-49.
30 Casanova JL, Conley ME, Seligman SJ, Abel L, Notarangelo LD. Guidelines for genetic studies in single patients: lessons from primary immunodeficiencies. J Exp Med 2014;211(11):2137-49.
31 Picard C, Al-Herz W , Bousfiha A Casanova JL , Chatila T , Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. J Clin Immunol 2015: 35(8):696-726.
32 Younger EM, Epland K, Zampelli A, Hintermeyer MK. Primary immuno-deficiency diseases: a primer for PCPs. Nurse Pract 2015;40(2):1-7.
33 Chapel H, Prevot J, Gaspar HB, Español T, Bonilla FA, Solis L, Drabwell J. Editorial Board for Working Party on Principles of Care at IPOPI. Primary immune deficiencies

- principles of care. Front Immunol 2014;5:627.
34 Abolhassani H, Asgardoon MH, Rezaei N, Hammarstrom L, Aghamohammadi A. Different brands of intravenous immunoglobulin for primary immunodeficiencies: how to choose the best option for the patient? Expert Rev Clin Immunol 2015;11(11):1229-43.
35 Jolles S, Orange JS, Gardulf A, Stein MR, Shapiro R, Borte M, Berger M. Current treatment options with immunoglobulin G for the individualization of care in patients with primary immunodeficiency disease. Clin Exp Immunol 2015;179(2):146-60.