REVIEW ARTICLE

Diagnostic Approach to Primary Immunodeficiency Disorders

M MADKAIKAR, A MISHRA AND K GHOSH

From Department of Pediatric Immunology and Leukocyte Biology, National Institute of Immunohaematology (ICMR), 13th Floor, NMS Bldg, KEM Hospital, Parel, Mumbai, India.

Correspondence to: Dr Manisha Madkaikar, National Institute of Immunohaematology, 13th Floor, NMS Bldg, KEM Hospital, Parel, Mumbai 400 012, India. madkaikarmanisha@icmr.org.in

Primary immunodeficiency disorders (PIDs) are a heterogeneous group of inherited disorders that affect different components of the immune system. There are more than 150 different disorders which have been described till date. Despite major advances in the molecular characterization of PIDs over the last 20 years, many patients remain undiagnosed or are diagnosed too late with severe consequences. Recognizing different clinical manifestations of PID is the first most important step. It should be followed by use of appropriate diagnostic tools from a vast number of investigations available. This review will focus on important presenting features of PID and laboratory approach for diagnosis of suspected cases of PID.

Key words: *Antibody deficiency, Phagocytic defects, Immune dysregulation, Primary immunodeficiency disorders, Severe combined immunodeficiency (SCID).*

Primary immunodeficiency disorders (PIDs) comprise more than 150 different disorders that affect the development, function, or both of the immune system [1]. In most cases, PIDs are monogenic disorders that follow a simple Mendelian inheritance; however, some PIDs are of more complex polygenic origin. All forms of PIDs are rare and have an overall prevalence of approximately 1:10,000 live births with the exception of IgA deficiency. However, a much higher rate is observed among populations with high consanguinity or among genetically isolated populations.

PIDs are classified into eight major categories according to the component of the immune system primarily involved [1]:

1. Combined T-cell and B-cell immunodeficiencies
2. Predominantly antibody deficiencies
3. Other well defined immunodeficiency syndromes
4. Diseases of immune dysregulation
5. Congenital defects of phagocyte number and function
6. Defects in innate immunity
7. Autoinflammatory disorders
8. Complement deficiencies.

In infants and children (early childhood), the immune system is not fully developed and they are also exposed to many pathogens as they mix with family members and other children in the nursery. Therefore recurrent infections are common in young children. Recurrent or persistent infection is the major manifestation of primary immunodeficiency (PID), though the pattern, infecting microorganisms and the severity of infections is usually different. While most children with recurrent infections have a normal immune system, it is important to recognize a child with an underlying PID from a normal child so that further investigations can be ordered selectively. Prompt and accurate diagnosis of PID not only helps to direct the most appropriate treatment, and predict prognosis, but also it is important for further genetic counseling for the family.

The treatment modalities for PID mainly include immunoglobulin replacement, antibiotics and bone marrow transplantation. Immunoglobulin replacement and judicious use of prophylactic antibiotics can prevent the significant end organ damage and improve long-term outcome and quality of life in many patients with PID if diagnosed early [2]. Hematopoietic stem cell transplantation is used for treating many of the severe immunodeficiencies. In centers specialized in treating these conditions, the survival and cure of the disease can reach up to 95%, depending on the condition of the patient at the time of treatment and the donor availability [3]. Thus it is important to recognize children with PID before significant end organ damage occurs to maximize the opportunity for successful treatment and a normal lifespan.

In this review we have highlighted the important clinical manifestations of PIDs including the pattern of infections which would alert the clinicians to suspect PID and the laboratory approach required for further evaluation of some common categories of PID.

RECOGNIZING CLINICAL MANIFESTATIONS OF PID

Careful clinical evaluation is crucial for recognition of patients with PID. It is important to know the presenting features and warning signs of PID in order to decide the need for further investigations. The European Society of Immunodeficiencies (ESID) has suggested 10 warning signs for suspicion of PID [4] (**Box 1**) [4,7].

Although this does not include comprehensive list of all signs and symptoms of PID, patients showing these signs must be evaluated further for an underlying PID. While evaluating such children, important clinical features like age at presentation, pattern of infection, non-infectious manifestations and family history should also be taken into consideration as these give an important clue to the underlying immune defect [5]. Though it is difficult to predict a specific PID on the basis of infections with particular organisms, they definitely provide an important clue to underlying immune defect (***Table* I**). The important distinct clinical manifestations of different categories of PID are discussed below.

Combined T and B Cell Deficiency

There are 22 different groups of diseases that have been included in this category [1]. Severe combined immunodeficiencies (SCID) like Adenosine deaminase (ADA) deficiency, purine nucleotide phosphorylase (PNP) deficiency, RAG1/2 deficiency, α chain deficiency, IL7Rα deficiency, and JAK3 deficiency and combined immunodeficiency (CID) like CD40 ligand deficiency (X-linked hyper IgM) are some of the common combined immunodefciencies. These patients usually present within first six months of life with failure to thrive, chronic diarrhea, persistent oral thrush, skin rash, pneumonia, and sepsis. Disseminated BCG infection is commonly seen in patients with SCID. Similarly, prolonged interstitial pneumonia of viral etiology such as parainfluenza virus or cytomegalovirus or *Pneumocystis jerovici* is also common in patients with combined immunodeficiency [6, 7].

Omenn syndrome is a rare autosomal recessive disease usually presenting in neonatal period, characterized by symptoms of SCID associated with other findings like erythroderma, lymphadenopathy, hepatosplenomegaly and eosinophilia. Most of the patients with PNP deficiency have neurological problems including developmental delay, hypertonia, spasticity, tremors, ataxia, retarded motor development, behavioral difficulties and varying degrees of mental retardation. Characteristic abnormality in ADA deficient SCID includes cupping at the end of the ribs demonstrated on a chest radiograph. They may also present with delayed development, deafness and seizures.

Box 1 Warning Signs for Suspicion of Primary Immuno-deficiency Disorders [4].

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

Lymphopenia is commonly seen with patients with SCID and requires further evaluation for specific diagnosis. However normal absolute lymphocyte count does not rule out combined immunodeficiency, thus further laboratory evaluation is required in case of strong clinical suspicion. Neutropenia is seen in many PIDs [8] including CD40L deficiency.

Predominant Antibody Deficiency

This category includes 6 groups of diseases [1] of which X-linked agammaglobulinemia (XLA) and common variable immunodeficiency (CVID) are the commonest. Patients with XLA typically present after 6-9 months of age when the level of protective maternal IgG starts going down [9]. Recurrent sinopulmonary infections due to *S. pneumonia* or *H. influenza,* otitis media, and septicemia are the most common clinical manifestations. Less common manifestations include enteroviral infections with resultant chronic meningitis, dermatomyositis, and rheumatoid like arthritis. Patients of CVID usually present later in life that is after 5 years though some may present as early as 2 years of age. There is also an increased risk of cancer in CVID cases predominantly with lymphoreticular tumors and some patients can also develop autoimmune diseases [10].

Other Well-defined PID

This category includes 9 different groups of diseases such as Ataxia telengiectasia (AT), DiGeorge syndrome, Wiskott-Aldrich syndrome (WAS) and hyper IgE syndrome (HIGE) [1]**.** Patients with AT or Nijmejen Breakage syndrome can present at the age of 6 months to 5 years with gait abnormalities or neurodevelopmental delay. Progressive cerebellar ataxia with discrete or pronounced telengiectasia involving the conjunctiva ears and sometimes face are the classical findings in ataxia-telengiectasia. Patients with DiGeorge syndrome present in neonatal period. This defect should be

TABLE I CLUES TO THE PRESENCE OF PRIMARY IMMUNODEFICIENCY

PID Category	*Infectious complications*	*Organisms*	*Diagnostic tests*
Combined T and B cell deficiency	Systemic viral infections, gastroenteritis	*Bacteria:* Pyogenic bacteria *Campylobacter Listeria*	*T cells:* Lymphocyte subsets: 1. T, Tc, Th, B , NK 2. DNT cells 3. Memory, naïve and activated T cell Specific cell surface antigen expressions: 1. CD132: 2. CD127 3. CD154 *Functional assays:* pSATA5 expression after stimulation pSTAT3 expression after stimulation T cell proliferation assays by CFSE Certain cytokine estimations: IL-10, IL-12 and INF g RBC ADA levels
		Viruses: All, especially, respiratory syncytial virus, EBV, parainfluenza type 3	
		Fungi:Candida, Aspergillus	
		Mycobacteria: Nontuberculous including BCG	
		Protozoa:Pneumocystis jiroveci, Toxoplasma gondii, Cryptosporidium parvum	
Antibody deficiency	Upper and lower respiratory tract, GI tract, skin infections, sepsis, meningitis	*Bacteria:S. pneumoniae, H. influenzae M. catarrhalis, P. aeruginosa, S. aureus N. meningitidis, M. pneumoniae*	*B cells:* 1. B cell numbers: CD19, CD79a, CD20 2. Intracellular Btk expression 3. Immunoglobulin estimation by nephelometry:Ig G, A, E,M Specific antibody responses
		Viruses: Enteroviruses	
		Protozoa: *Giardia lamblia*	
Phagocytic defects	Respiratory tract, Liver or lung abscesses, GI diseases, urinary tract problems	*Bacteria: S.aureus, P. aeruginosa Nocardia asteroids, S. typhi*	*Phagocytic Functions:* 1. CD18, CD11 expression: 2. DHR 3. NBT
		Fungi: Candida, Aspergillus	
		Mycobacteria: Nontuberculous including BCG	
Complement deficiency	Meningitis, systemic bacterial infections	*Bacteria:* Streptococci, *H. influenzae, Neiserria*	Functional hemolytic assay (CH50 and AH50 assays) and serum concentration measurement for complement components
		Viruses: CMV, HSV	

suspected in patients with cardiac defects with hypoplastic thymus, hypocalcemia and facial dysmorphism. Eczema in infancy and recurrent staphylococcal skin boils and pneumonia with pneumatocele formation are the commonest presenting manifestations of HIES due to STAT3 defect [11]. Patients with HIES due to DOCK8 deficiency usually present with disseminated moluscum contagiosum (***Fig.1***) or

disseminated viral warts [12]. Autosomal dominant HIES is commonly associated with multiple connective tissue and skeletal abnormalities including scoliosis, hyper extensibility, pathologic fractures, retained primary dentition, craniosynostosis, and vascular abnormalities [13]. Central nervous system abnormalities are common in HIES. Asymptomatic cerebral T2-weighted hyper-intensities, increased prevalence of lacunar infarcts, and increased Arnold Chiari 1 malformations are seen in the brain MRI of many patients [14].

WAS patients present with eczema, petechiae and recurrent sino-pulmonary manifestations [15]. The incidence of EBV associated lymphoma is also high in these patients. Thrombocytopenia with low mean platelet volume gives important clue for diagnosis of WAS.

Phagocytic defects

This category of PID includes 5 groups of diseases [1] of which leukocyte adhesion deficiency-I (LAD-I), chronic granulomatous disease (CGD) and severe congenital neutropenia (SCN) are some of the common diseases. Patients with phagocytic defects usually present in neonatal period. Delayed separation of umbilical cord beyond 2 weeks along with omphalitis is suggestive of a neutrophil disorder like LAD-I [16], SCN or CGD. Patients with LAD-II have severe mental retardation, short stature, a distinctive facial appearance and the rare Bombay (Hh) phenotype. Eczematous rash with deep seated abscesses is associated with CGD. Infections due to *S. aureus*, *Burkholderia cepacia* and fungal infections (mainly *Aspergillus*) are common in CGD [17].

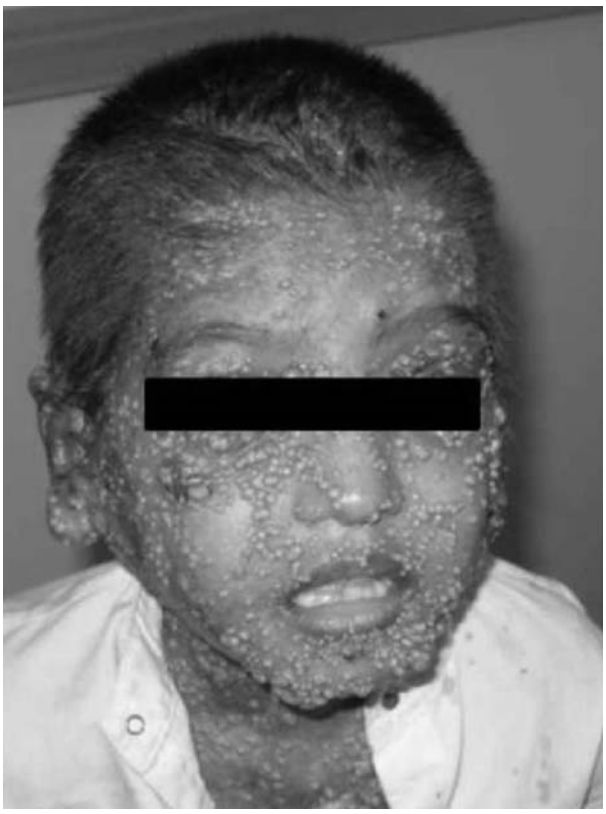

FIG. 1 *Patient with HIES due to DOCK8 deficiency with disseminated Molluscum contagiosum.*

Disseminated atypical mycobacterial infection or BCGiosis or recurrent salmonella infection in an otherwise well grown individual leads to suspicion to Mendelian susceptibility to mycobacterial diseases (MSMD) due to type-I cytokine defects [18]. Persistent neutrophilia even in the absence of active infection is a common feature of LAD-I. In severe congenital neutropenia child has persistently low absolute neutrophil counts (ANC) with elevated monocytes and eosinophils counts. Cyclic neutropenia patients present with drop in ANC every 3-4 weeks with fever, infections and mouth ulcers.

Diseases of immune dysregulation

Four groups of diseases are included in this category [1]. Familial Hemophagocytic Lymphohistiocytosis (FHL) that includes Perforin deficiency, UNC13D (Munc13-4) deficiency, Syntaxin 11 deficiency and STXBP2 (Munc 18-2) deficiency and Autoimmune Lymphoproliferative syndrome (ALPS) are the most common groups of diseases in this category. Patients with diseases of perforin defect usually present at less than six months of age. Patients with Autoimmune Lymphoproliferative syndrome (ALPS) present at the median age of around 2 years with chronic nonmalignant lymphadenopathy, splenomegaly and immune cytopenias [19]. Mucocutaneous albinism is seen with patients with Griselli syndrome and Chediak-Highashi syndrome [20].

Patients with hemophagocytic lymphohistiocytosis (HLH) (either familial or associated with Chediak-Higashi or Griscelli syndrome-II, X-linked lymphoproliferative syndrome) are associated with varying degrees of cytopenias with hemophagocytosis seen either in the bone marrow or rarely in the peripheral blood. Patients with ALPS generally have elevated ALC with recurrent non-malignant lymphadenopathy and spleno-hepatomegaly. Patients with Evan's syndrome should be evaluated for underlying ALPS as high proportion of these cases have *FAS* gene mutation [21]. Autoimmune cytopenias are also commonly seen in patients with immune dysregulation, polyendocrinopathy, enteropathy or X-linked (IPEX) syndrome. Hematolymphoid malignancies are common with certain PID. Patients with ALPS are at a higher risk of developing the Non-Hodgkin and Hodgkin lymphoma. EBV-associated lymphoma is common in patients with XLP.

Complement deficiency

Patients with complement deficiency present later in life usually after 5 years of age. Autoimmune disease and pyogenic infections are often associated with a deficiency of early components (complements 1-4) of the classic pathway. Terminal complement component deficiencies (complements 5-9) have increased susceptibility to serious infections from *Neisseria* species [10]. Complications such as recurrent pneumonia, meningitis, and peritonitis are seen in complement 3 deficiency.

LABORATORY APPROACH TO PATIENTS WITH PID

With wide array of assays being available for evaluation of immune system, it becomes difficult to choose an investigation to be performed. The investigations are largely guided by the clinical presentation of the patient, the suspected immune defect and the results of initial laboratory evaluation.

The most useful first-line immunological investigations include a complete blood count with a differential count on the leucocytes and MPV, lymphocyte subset analysis, serum immunoglobulin levels and Nitroblue Tetrazolium test (NBT). The panel of antibodies used for these purpose includes CD3, CD4, CD8, CD56/16, CD19 and HLA-DR. It is aimed at measuring the absolute and relative number of : B cells (CD19+), T cells (CD3+), T-helper cells (Th, CD3+/CD4+), T-cytotoxic cells (Tc, CD3+/CD8+), Natural Killer (NK) cells (CD3-/CD56+/CD16+), and Activated T cells (CD3+/HLA-DR+).

It is very important to note that the total lymphocyte numbers and T lymphocyte subsets are age-dependent, being markedly increased in newborns and young infants and decreasing with age. In infants below 4 months of age, a CD4 count of <1000/mm^3 is generally associated with impaired cellular immunity, whereas the corresponding value is <500/mm^3 in children over 2 years of age and in adults [22]. Immunosuppressive therapies like steroids also significantly alter the values of T and B cell subsets and should be interpreted carefully.

The results of the initial tests usually give an important clue to the underlying immune defect. Patients with low T cell counts are likely to have combined T and B cell defects (CID). Patients with low or absent B cell and low Immunoglobulin levels with normal T cell fall in the category of predominantly antibody deficiency. Patients with abnormal neutrophil count or abnormal neutrophil function suggest defects in the phagocytic system. However, under these broad categories, there are many subcategories or genetic defects and one needs advanced laboratory tests available only at specialized centers to come to a specific diagnosis. Details of evaluation follow:

Suspected Combined T and B-cell Immunodeficiency

Lymphocyte subset analysis is abnormal in most cases of SCID and in many cases of CID. SCID comprises of a group of inherited disorders that characteristically show abnormalities in T, B, and natural killer (NK) cell function. These are categorized broadly as T+ SCID and T-SCID depending on presence or absence of the T cells.

There are many genetic defects which can lead to T-SCID phenotype [23]. The B cells and NK cells count in these patients give an important clue to the underlying molecular defects (***Fig.* 2**). However, there is significant overlap between these categories and hence specialized tests like CD132 and CD127 expression, functional studies like pSTAT5 activation in lymphocytes after IL-2 stimulation, estimation of enzymes like ADA and PNP in RBCs, radiation sensitivity test, etc. are required for specific diagnosis.

Patients with normal T cell numbers can still have CID. This is usually seen with patients with Omenn syndrome, MHC-I or MHC-II deficiency, ZAP 70 deficiency, etc. These patients can be evaluated by doing T cell proliferation assays (for evaluation of T cell function), expression of HLA-DR on T and B cells (for MHC class-II expression) and T cell receptor (TCR) V-beta repertoire analysis (for assessment of diversity of immune response).

Suspected B-cell Defect

Patients with suspected B cell defects require estimation of B cell numbers (CD19, CD20 and CD79a), and serum immunoglobulin levels (IgG, IgA, IgM, IgE and IgG subclasses). Patients with absent B cells and markedly reduced Ig are suggestive of agammaglobulinemia which

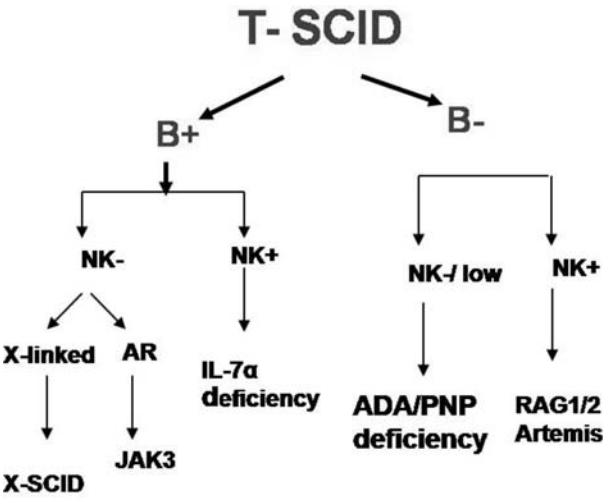

FIG. 2 *Evaluation of patients with T- SCID.*

can be X-linked (XLA) or autosomal recessive agammaglobulinemia. Patients with XLA will have absent or reduced expression of protein Bruton Tyrosine Kinase (BTK) with carrier mothers showing mosaic pattern. Patients with reduced Ig with normal to low B cells with abnormal specific antibody responses suggest common variable immunodeficiency (CVID). Patients with Hyper IgM syndrome (HIGM) have markedly low IgG and IgA with normal to elevated IgM levels. They can be further evaluated by studying expression of CD40 and CD40L (CD154) expression on B cells and T cells respectively. Patients with X-linked HIGM will have abnormal CD 154 expression on T cells after stimulation and carrier mothers will show mosaic pattern.

Some patients with these disorders may have normal or only modestly reduced immunoglobulin levels; therefore, the best approach for confirming a diagnosis of an antibody-deficiency disorder is the measurement of serum specific antibody titers (usually IgG) in response to vaccine antigens. This approach involves immunizing a patient with protein antigens (e.g., tetanus toxoid) and polysaccharide antigens (e.g., pneumococcus) and assessing pre- and post-immunization antibody levels. In many PIDs, antibody responses to these antigens are diminished or even absent.

Suspected Phagocytic Defects

In a patient with suspected phagocytic defect one must look at the absolute neutrophil count (ANC). A patient with low ANC with early neonatal presentation is suggestive of severe congenital neutropenia (SCN). Characteristically, there is marked monocytosis with levels often two to four times that of normal. There may be associated anemia and mild thrombocytosis attributable to chronic inflammation. Bone marrow examination shows the presence of early precursor cells but very few mature cells beyond the promyelocyte stage or 'promyelocyte arrest'. Patients with cyclic neutropenia have oscillations of neutrophil count with a periodicity of around 21 days. At the nadir, neutrophil counts are generally less than 0.2×10^9 per L for 3-5 days, after which they rise rapidly to levels near the lower limit of normal, about 2×10^9 per L. Both SCN and cyclic neutropenia commonly result from mutations in neutrophil elastase gene (*ELA-2*).

Patients with suspected CGD have normal or elevated ANC and can be diagnosed by NBT and DHR test. These tests can also detect carrier mothers in X-linked CGD. Final confirmation of underlying defect can be done by studying the intracellular expression of gp91 for X-CGD and p22, p67 or p47 for autosomal recessive CGD followed by molecular analysis of the affected gene. Patients with LAD-I can be easily diagnosed by flowcytometric analysis of CD18, CD11a, CD11b and CD11c expression on peripheral blood leukocytes.

Suspected Immune Dysregulation

There are two important groups of disorders in this category.

Familial Hemophagocytic Lymphohistiocytosis (HLH): HLH can result from secondary causes like infections, malignancy, rheumatic diseases or toxins or may be due to inherited genetic defect leading to impaired NK cell function. It is also important to differentiate primary HLH from secondary HLH as the patients with primary HLH are more likely to relapse after therapy and require hematopoietic stem cell transplantation for long term survival. The diagnosis of HLH is often difficult due to the rarity of the disease and lack of a specific laboratory test, resulting in under diagnosis. In an attempt to overcome these difficulties, the FHL study group of the Histiocyte Society has proposed diagnostic guidelines for HLH (***Table* II**) [24]. The diagnosis of HLH is based on clinical and laboratory criteria which involve complete hemogram, bone marrow aspiration studies, liver function tests, estimation of S bilirubin, S ferritin, S triglyceride, S

TABLE II DIAGNOSTIC CRITERIA FOR HLH PROPOSED BY HISTIOCYTE SOCIETY

1. Molecular diagnosis of hemophagocytic lymphohistiocytosis (HLH) or X-linked lymphoproliferative syndrome (XLP).

   OR

2. at least 3 of 4:
   a. Fever ≥38.5°C
   b. Splenomegaly
   c. Cytopenias (minimum 2 cell lines reduced)
      Hemoglobin < 9g/dL (in infants < 4 weeks;
      Hemoglobin < 10g/dL; Platelets < 100×10^3/mL;
      Neutrophils < 1×10^3/mL
   d. Hepatitis
3. And at least 1 of 4:
   a. Hemophagocytosis
   b. Ferritin > 500ng/mL
   c. ↑sIL2Rα (age-based)
   d. Absent or very low NK function
4. Other results supportive of HLH diagnosis:
   a. Hypertriglyceridemia (>265 mg/dL)
   b. Hypofibrinogenemia (<150 mg/dL)
   c. Hyponatremia

Source: ASH Education Book January 1, 2009 vol. 2009 no. 1 127-131.

fibrinogen and sCD25 levels [25]. Further evaluation using NK cell cytotoxicity assay, Perforin expression studies, Granule release by NK cells and SAP and XIAP expression on lymphocytes and MUNC 13-4 and SYNTAXIN-11 by western blot help significantly in diagnosis of genetic HLH.

Autoimmune lymphoproliferative syndromes (ALPS): Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of lymphocyte homeostasis characterized by non-malignant lymphoproliferation autoimmunity mostly directed toward blood cells and increased risk of lymphoma. If ALPS is suspected based on clinical findings, initial laboratory evaluation includes flow cytometric analysis of peripheral blood circulating TCR ab^+ DNT cells and estimation of serum B12, soluble FAS ligand (sFASL), interleukin (IL) -10 and IL-18 levels. The recommended percentage of TCR ab^+DNT cells required for a diagnosis is greater than or equal to 1.5% of total lymphocytes or 2.5% of T lymphocytes in the setting of normal or elevated lymphocyte counts. The presence of elevated TCR ab^+ DNT cells coupled with high serum or plasma levels of either IL -10, IL-18, (sFASL) or vitamin B12 can accurately predict the presence of germ line or somatic *FAS* mutations [26].

Suspected Complement Deficiency

In patients with suspected complement deficiency, initial evaluation is done with the CH50 (which tests the classical and final lytic components except C9) and AH 50 (which tests alternative and final lytic pathways) assays. These tests should be done in laboratories with considerable experience of these assays. To avoid misinterpretation due to the possible effects of complement consumption by immune complex formation, it is advisable that the assays be performed when the patient has completely recovered from immune complex disease or infection. Both the tests require blood to be taken atraumatically and serum be separated within 1 hour and stored at -70^0C. If either of these screening tests identifies failure of a complement pathway on two occasions, the specific component defect should be determined.

CONCLUSION

Diagnosis of specific PID from a large spectrum of disorders requires expertise in clinical and laboratory evaluation. Wide array of assays are available for evaluation of immune system which help immensely in the diagnosis of PIDs. Knowledge of clinical presentation of these disorders, correct interpretation of initial results of immunophenotyping of lymphocytes is essential for choosing the appropriate test for specific diagnosis. There is very little data available from India on PID. Being a country with the second largest population in the world, we are likely to have large number of patients with PIDs. Recognizing a suspicious case of PID at a regional hospital level is important to ensure timely referral to a specialized centre for diagnosis and treatment for these patients. We need to create awareness about these disorders and possibly establish more centers with diagnostic facilities for their evaluation.

Contributors: MM has conceived and written the manuscript. GK revised the manuscript for important intellectual content. MA compiled the data and helped in manuscript writing. The final manuscript was approved by all authors.
Funding: None; *Competing interests*: None stated.

REFERENCES

1. Al-Herz W, Bousfiha A, Casanova JL, Chapel H, Conley ME, Cunningham-Rundles C, *et al*. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. Frontiers in Immunology. 2011;2:54.
2. Piguet D, Tosi C, Luthi JM, Andresen I, Juge O. Redimune ((R)) NF Liquid, a ready-to-use, high-concentration intravenous immunoglobulin therapy preparation, is safe and typically well tolerated in the routine clinical management of a broad range of conditions. Clin Exp Immunol. 2008;152:45-9.
3. Notarangelo LD, Forino C, Mazzolari E. Stem cell transplantation in primary immunodeficiencies. Curr Opin Allergy Clin Immunol. 2006;6:443-8.
4. Subbarayan A, Colarusso G, Hughes SM, Gennery AR, Slatter M, Cant AJ, *et al*. Clinical features that identify children with primary immunodeficiency diseases. Pediatrics. 2011;127:810-6.
5. Slatter MA, Gennery AR. Clinical immunology review series: an approach to the patient with recurrent infections in childhood. Clin Exp Immunol. 2008;152:389-96.
6. Winkelstein JA, Marino MC, Ochs H, Fuleihan R, Scholl PR, Geha R, *et al.* The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. Medicine (Baltimore). 2003;82:373-84.
7. Berrington JE, Flood TJ, Abinun M, Galloway A, Cant AJ. Unsuspected *Pneumocystis carinii* pneumonia at presentation of severe primary immunodeficiency. Arch Dis Child. 2000;82:144-7.
8. Bouma G, Ancliff PJ, Thrasher AJ, Burns SO. Recent advances in the understanding of genetic defects of neutrophil number and function. Br J Haematol. 2010;151:312-26.
9. Wood P, Stanworth S, Burton J, Jones A, Peckham DG, Green T, *et al.* UK Primary Immunodeficiency Network. Recognition, clinical diagnosis and management of patients with primary antibody deficiencies: a systematic review. Clin Exp Immunol. 2007;149:410-23.
10. Zi Yin E, Frush DP, Donnelly LF, Buckleyl RH. Primary immunodeficiency disorders in pediatric patients: Clinical

features and imaging findings. Am J Roentgeno. 2001;176:1541-52.
11. Woellner C, Gertz EM, Schäffer AA, Lagos M, Perro M, Glocker EO, *et al*. Mutations in STAT3 and diagnostic guidelines for hyper-IgE syndrome. J Allergy Clin Immunol. 2010;125:424-32.
12. Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, *et al*. Combined immunodeficiency associated with DOCK8 mutations. N Engl J Med. 2009;361:2046-55.
13. Buckley RH. The Hyper-IgE syndrome. Clin Rev Allergy Immunol. 2001;20:139-54.
14. Freeman AF, Collura-Burke CJ, Patronas NJ, Ilcus LS, Darnell D, Davis J, *et al*. Brain abnormalities in patients with hyperimmunoglobulin E syndrome. Pediatrics. 2007;119:e1121-5.
15. Albert MH, Notarangelo LD, Ochs HD. Clinical spectrum, pathophysiology and treatment of the Wiskott-Aldrich syndrome. Curr Opin Hematol. 2010. Nov 11. [Epub ahead of print]
16. Madkaikar M, Currimbhoy Z , Gupta M, Desai M, Rao M. Clinical profile of leukocyte adhesion deficiency. Indian Pediatr. 2011;49:1-4.
17. Holland SM. Chronic granulomatous disease. Clin Rev Allergy Immunol. 2010;38:3-10.
18. Al-Muhsen S, Casanova JL. The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. J Allergy Clin Immunol. 2008;122:1043-51.
19. Madkaikar M, Mhatre S, Gupta M, Ghosh K. Advances in autoimmune lymphoproliferative syndromes. Eur J Haematol. 2011;87:1-9.
20. Dessinioti C, Stratigos AJ, Rigopoulos D, Katsambas AD. A review of genetic disorders of hypopigmentation: lessons learned from the biology of melanocytes. Exp Dermatol. 2009;18:741-9.
21. Teachey D, Manno CS, Axsom KM, Andrews T, Choi JK, Greenbaum BH, *et al*. Unmasking Evans syndrome: T-cell phenotype and apoptotic response reveal autoimmune lymphoproliferative syndrome (ALPS). Blood. 2005;105:2443.
22. Melaranci C, Ciaffi P, Zerella A, Martinelli V, Cutrera R, Plebani A, *et al*. T cell subpopulation in paediatric healthy children: age-normal values. J Clin Lab Immunol. 1992;38:143-9.
23. Walshe D, Gaspar HB, Thrasher AJ, Cale CM, Gilmour KC. Signal transducer and activator of transcription 5 tyrosine phosphorylation for the diagnosis and monitoring of patients with severe combined immunodeficiency. J Allergy Clin Immunol. 2009;123:505-8.
24. Henter J, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, *et al*. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2006;48:124-31.
25. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. Eur J Pediatr. 2007;166:95–100.
26. Caminha I, Fleisher TA, Hornung RL, Dale JK, Niemela JE, Price S, *et al*. Utilizing biomarkers to predict the presence of FAS mutations in patients with features of the autoimmune lymphoproliferative syndrome. J Allergy Clin Immunol. 2010;125:946-9.