Spotlight on Primary Immune Deficiency Disorders

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August 8, 2019

I am extremely happy to learn that the Institute of Maternal and Child Health, Government Medical College, Kozhikode is organizing "CAREPID: Spotlight on Primary Immune Deficiency Disorders". Though Kerala has brought down Infant Mortality Rate almost on par with that of developed countries there still remain a large number of neglected and rare diseases that need our immediate attention. It is estimated that there are at least 34 such diseases that we have to focus our attention immediately. Primary Immune Deficiency Disorders, Inborn Errors of Metabolism and Lysosomal Storage Diseases and Congenital Heart Disease are a few of such diseases not presently being given proper attention due largely due to lack of awareness among the policy makers and the medical profession.

The health department has already taken initiative for the early diagnosis and management of some of these disorders. The Public Health Laboratory at Thiruvananthapuram and at their three regional laboratories are presently doing newborn metabolic screening of four parameters: Congenital Hypothyroidism, Congenital Adrenal Hyperplasia, G6PD Deficiency and Galactosaemia. Anomaly scan training is being promoted for the early detection and management of Congenital Heart Diseases. Several such initiatives are necessary with regards to other conditions also.

I hope that the deliberations in this conference will pave the way for the early diagnosis and more focused and comprehensive management of Primary Immune Deficiency Disorders in our state.

Wishing the conference all success,
Yours Sincerely,

B.Ekbal

Prof. VR Rajendran
Principal,
Govt. Medical College, Kozhikode

August 10, 2019

MESSAGE

It is being increasingly recognized that primary immune deficiency disorders are an important cause of mortality and morbidity. Children often present late, and sometimes, organ damage has already occurred. Improved awareness among medical professionals will certainly help in improving outcomes, through early diagnosis and referral.

It is a matter of great pride that Government Medical College, Kozhikode now offers several immunological and molecular diagnostic tests for the diagnosis of these rare disorders, and also has facilities for antenatal diagnosis and genetic counseling.

I wish all success to the organizing team for the CME program being conducted in association with the CSIR Institute of Genomics and integrative Biology, Foundation for Primary Immunodeficiencies and Indian Academy of Pediatrics.

I am sure that such programs will benefit students, practicing medical professionals and patients alike and hope that this is only the beginning!

FPID Foundation for Primary Immunodeficiency Diseases

August 8, 2019

Dr.Geeta Govindaraj
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Dear Dr. Govindaraj:

I congratulate you for organizing this CME on Primary Immunodeficiency Diseases (PID).

Primary immunodeficiency diseases, once considered very rare disorders, are not so rare. They are four times more common than hemophilia, five times more common than cystic fibrosis, and almost as common as multiple sclerosis, yet very few clinicians know about them. There are more than 378 PIDs and more than 400 gene mutations responsible have been discovered. In infants and children, PIDs are immunological emergency that require early diagnosis and treatment with immunoglobulins and bone marrow transplantation. PIDs are as common in adults as in children; however, not as serious as in children. In approximately 25% of adult patients, it takes more than 20 years to diagnose PID. This is mainly because of lack of awareness and knowledge among clinicians about PIDs. Majority of patients with PIDs present with recurrent infections usually by common microorganisms; however, a subsets of patient may present with non-infectious manifestations related to hematology-oncology, gastroenterology, ENT, pulmonary, and rheumatological diseases.

In 2010, we started Foundation for Primary Immunodeficiency Diseases (FPID) with the mission to increase awareness among general public and clinicians, educate clinicians and scientists, improve early diagnosis, and support treatment in India. Currently, we have 8 FPID Centers across India. Two of these centers are designated as Center of Excellence, where prenatal, neonatal, and early post-natal diagnosis of PID can be made. There are eight centers in India, where bone marrow transplantation for PID are currently performed.

I am confident that your CME course will contribute to an increase awareness among fellow clinicians, including pediatricians, physicians, and clinicians from various subspecialties, and to educate them about clinical manifestations, early diagnosis, and treatment of PIDs.

I wish you a successful CME course.

Yours Sincerely,

Sudhir Gupta, MD, Ph.D., D.Sc (h.c.), MACP, FRCP (C), FRCP (Lond)
Co-Founder, President and Director, and Chairman of the Board of Directors
Foundation for Primary Immunodeficiency Diseases
Professor of Medicine, Pathology & Laboratory Medicine, and Microbiology & Molecular Genetics
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What you will read in this digital Booklet

Primary immune deficiency disorders – An introduction and clinical cues to diagnosis

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Primary immunedeficiency disorders (PIDs) are rare inherited disorders resulting from developmental or functional derangements in the immune system. The preferred term at present is 'Inborn errors of immunity'. There is a paucity of studies on these rare disorders from this part of the country, and there is evidence that they are more common than they are in the West. New achievements in this field have been possible due to collaborative efforts, improved immunologic techniques, and use of next generation sequencing technology.

With the reduction in the healthcare burden of infectious diseases in children due to immunization and improved standards of living, the emphasis is gradually shifting towards children who are at risk of death or disability due to their enhanced susceptibility to infections. The availability of treatment options like bone marrow and stem cell transplantation, which were once considered to be out of reach, and of life saving prophylactic therapies like intravenous immunoglobulin, has given added impetus to arriving at an early diagnosis.

Before one considers the diagnosis of a PID, it is mandatory to rule out causes of secondary immunocompromised states like HIV

infection, diabetes mellitus, malignancy, nephrotic syndrome or exposure to drugs like corticosteroids or chemotherapeutic agents. It is also important to keep in mind that young children get frequent upper respiratory infections, usually viral in aetiology after starting school. Although unusual infections either in terms of frequency, severity, organ involvement or offending organisms are the most common manifestations of PIDs in children, autoimmunity, auto- inflammation, allergy or malignancy may be a predominant feature. PIDs have been classified based on the predominant defect into nine categories by the International Union of Immunological Societies (IUIS).

Diseases of immune dysregulation include hemophagocytic lympho-histiocytosis and autoimmune lymphoproliferative syndrome. Auto-inflammatory disorders are being increasingly recognized and include Hyper IgD syndrome, Familial Mediterranean fever, Blau syndrome and the cryopyrinopathies. Autoinflammatory bone diseases like Chronic Recurrent Multifocal Osteomyelitis are also known to occur.

JMF* Warning signs for PID in children

- Four or more new ear infections within one year
- Two or more sinus infections within one year
- Two or more months on 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 oral thrush or fungal infections on the skin
- Need for intravenous antibiotics to clear infections
- Two or more deep seated infections including septicemia
- A family history of PID

*Jeffrey Modell Foundation

The above-mentioned warning signs are used in India since there have not been any country specific alternatives. It is suggested that children with two warning signs undergo screening after prioritization of lab tests based on clues from the history and physical examination. However, it should be emphasized that it is always wise to screen for a PID if there is a positive family history, even when the child is still asymptomatic.

Age at presentation

Although T cell and phagocytic defects commonly present during the first three months of life, B cell defects usually become symptomatic after six months of life when maternal IgG levels have waned. Adult onset PIDs are also being recognized increasingly, including CVID and some forms of CGD.

Gender

X linked defects should be strongly suspected in a male child with a presentation compatible with disorders including SCID (common gamma chain defect), XLA, WAS, XLP, XHIGM and so on, especially in the presence of a positive family history.

Family history

A carefully drawn pedigree chart helps ascertain the inheritance pattern in several cases, especially if there are other family members affected.Care should be taken to ask whether the parents are in fact the biological parents , and no assumptions should be made.Fetal loss and deaths of other family members should be recorded and the cause of death ascertained. Records of siblings who died should be scrupulously examined to reveal tell tale signs of a PID that was missed.

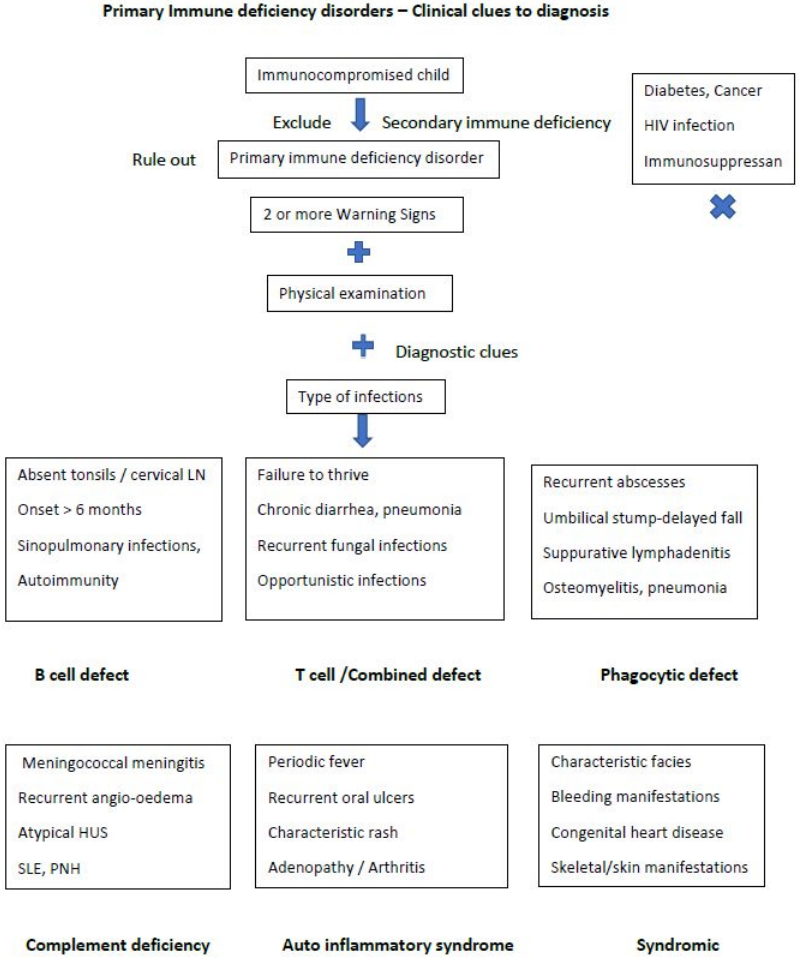
Physical examination

A meticulous physical examination would focus on the following clinical features, which could help to direct the sequence or priority of investigations in a child:

Facial dysmorphism.	DiGeorge syndrome, Hyper IgE syndrome
Absent tonsils / non - palpable lymph nodes	XLA, SCID
Skin lesions in newborns, infants or children	SCID (Omenn syndrome), IPEX, WAS
Bleeding manifestations with / without skin lesions	WAS, Chediak Higashi syndrome, ALPS
Depigmentation of skin, hair or eyes	Chediak Higashi syndrome, Griscelli syndrome
Gingivitis or oral ulcers	LAD, HIGM, CGD
Generalized lymphadenopathy and / or hepatosplenomegaly	ALPS, XLPS, MSMD
Well recognized syndromes	WAS, CHS, HIES, Dyskeratosis congenita

Anthropometry often gives a clue as to the type of PID one is dealing with eg. Severe failure to thrive is a hallmark of T cell deficiency, while short limbed dwarfism with increased upper: lower segment ratio would suggest cartilage – hair hypoplasia

along with other stigmata.Short trunk dwarfism would suggest associated spondylo epiphyseal dysplasias.



In Hyper IgE syndrome, facial features usually become manifest during adolescence and give the child the characteristic 'old man appearance'. Children with DiGeorge syndrome may not have the characteristic facial features, and in fact may come to attention

more often due to congenital heart disease or hypocalcemic seizures, rather than with immune deficiency.

Persistent oral thrush or cutaneous fungal infections should alert one to the possibility of immune deficiencies that predispose to these infections like those with T cell or combined immune deficiency, STAT 1 or CARD 9 mutations or APECED.
Dental abnormalities include retained primary teeth in the Hyper IgE syndrome and notched teeth in ectodermal dysplasia (anhidrotic or hypohidrotic) with immune deficiency.

Infections in PIDs vs Immune defect

T cell	**B cell**	**Phagocyte**
Common Gram positive and negative bacteria Mycobacterium	Pneumococcus, S. aureus H. influenzae	E.coli, Klebsiella, Nocardia, Pseudomonas Listeria, Salmonella, Burkholdaria
Toxoplasma, P. carinii Cryptosporidium, Giardia	Giardia, Cryptosporidia	
CMV, HSV, EBV, RSV. Enterovirus, VZV	Enterovirus	

Candida, Histoplasma		Candida, Aspergillus

Encapsulated bacteria like H. influenzae and S. pneumoniae are the common organisms in children with B cell defects. However, these children are also highly susceptible to enteroviral infections and should not receive OPV, which should also be avoided in household contacts.

T cell and combined immunodeficiencies put children at risk of a multitude of infections due to bacteria, fungi, viruses and opportunistic pathogens. Some organisms are 'signature or sentinel pathogens' and help zero in on the disease like Aspergillus in CGD.

Infections with atypical or environmental mycobacteria should make one suspect the disorder of the IFN gamma receptor pathway, Mendelian Susceptibility to Mycobacterial Disease.

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USEFUL WEBSITES

1.ISPID: The Indian Society for Primary Immune Deficiency
ispid.org.in
2.ESID - European Society for Immunodeficiencies
https://esid.org
3.Clinical Immunology Society -
cis.clinimmsoc.org
4.Asia Pacific Society for Immunodeficiencies -
paed.hku.hk/apsid
5.LASID Latin American Society for Immunodeficiencies
https://www.lasid.org

Dysmorphic features as clues to the diagnosis of Primary Immunodeficiency

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Primary immunodeficiencies are a group of conditions which are individually rare, but collectively somewhat common, the diagnosis of which are missed frequently. Delayed diagnosis can be life threatening. There are several clinical clues to the diagnosis by which we can even have a diagnosis as accurate as molecular diagnosis. At the same time many common genetic conditions have immunodeficiency as one of the manifestations.

Immunodeficiency in genetic syndromes can be due to various factors.

1.Mutation in a particular gene can cause both dysmorphic features and immune dysfunction, both related to the function of that particular gene.

2. Contiguous gene deletions as occurring when two genes are closely linked, one regulating immune function and the other regulating an organ structure or function.

3.Insult in crucial period of embryological development can affect different systems that develop at that particular time.

4.Exposure to acidosis or some toxic products as occurring in some of the IEMs affect proper functioning of the immune system.

List of some of the conditions with PID and dysmorphic features are

1.Syndromes associated with abnormalities of chromosome number or structure
(a)Trisomy 21
(b)DiGeorge syndrome
(c)Wolf Hirschhorn syndrome
(d)Turner syndrome
(e)Partial deletion of chromosome 18

2.Syndromes associated with growth deficiency
(a)Skeletal dysplasias
- I Cartilage Hair Hypoplasia
- II Short limbed skeletal dysplasias with immunodeficiency
- III Kenney Caffey syndrome/ Sanjad Sakati syndrome

(b) proportionate short stature
- I XLA with GH Deficiency
- II CHARGE association
- III Kabuki syndrome
- IV Rubinstein Taybi syndrome

3.Syndromes associated with skin manifestations
(a)Griscelli syndrome
(b)Incontinentia pigmenti
(c)Ectodermal dysplasia with ID
(d)Dyskeratosis congenita

(e)Acrodermatitis enteropathica
(f)Netherton syndrome
(g)Xeroderma pigmentosum

4.Syndromes with neurologic dysfunction
(a)Myotonic dystrophy
(b)Ataxia telangiectasia
(c)Leukocyte adhesion Defect type II

5.Syndromes with hematologic manifestation
(a)Wiskott-Aldrich syndrome
(b)Chediak Higashi syndrome
(c)Omenn syndrome
(d)Shwachman syndrome
(e)Pearson syndrome

6.Syndromes with ID as an associated feature
(a)Schwartz Jampel syndrome
(b)Beckwith Weideman syndrome
(c)Zellweger syndrome
(d)Menke syndrome
(e)Pseudo achondroplasia
(f)Smith Lemli Opitz syndrome

7.Syndromes with chromosome instability/abnormal DNA repair
(a)Bloom syndrome
(b)Fanconi anemia
(c)Ataxia Telangiectasia

Trisomy 21: This is the commonest chromosomal abnormality we see. They are prone to recurrent infections, especially respiratory. Although most individuals do not have clear immune

dysfunction, a number of immunologic abnormalities have been noted. Decreased B-cell number and low specific antibody response have been reported. Increased IgG and decreased IgM levels may occur during late childhood and adolescence. The thymus may be small with marked thymocyte depletion and an increased number of Has- sall's corpuscles.Proliferation in response to PHA and allo antigens, delayed cutaneous hypersensitivity response, and T cell-mediated killing is variably reduced. Total NK cell number is increased but the activity is decreased. Phagocyte number is normal, but chemotaxis and oxidative metabolism, and hence killing, are impaired.There is an increased incidence of autoimmune conditions.

DiGeorge syndrome: Many patients have a history of recurrent infection. Thymic hypoplasia is associated with DiGeorge syndrome. Overall, 77% of patients with the 22q11 deletion were immunocompromised. Impaired T-cell production was present in two-thirds of patients, and 23% had humoral defects, 19% had abnormal T-cell function, and 13% had IgA deficiency. In addition, a few patients showed significant improvement in T-cell pro- duction during early childhood. The severity of the immunodeficiency does not correlate with any specific clinical feature, and immunodeficiency was not limited to those with "classic" DiGeorge sequence.

Wolf Hirschhorn syndrome: Patients have frequent episodes of respiratory infections, due in part to recurrent aspiration, but antibody deficiencies are also common. Immune defects include common variable immunodeficiency, IgA and IgG2 subclass deficiency, IgA deficiency, and impaired polysaccharide responsiveness. T-cell immunity is normal.

Turner syndrome: The syndrome is associated with an increased risk for upper respiratory and ear infections, autoimmunity, and occasionally neoplasia. IgG, IgM, and/or IgA levels may be abnormal. Decreased T-cell number with poor response to PHA, absent delayed cutaneous hypersensitivity reactions, and common variable immunodeficiency occasionally occur.

Cartilage Hair Hypoplasia: Characterized by short-limb dwarfism, fine sparse hair, and a cellular immune defect. Metaphyseal dysplasia (flared, scalloped, and sclerotic metaphyseal ends) most frequently affects the lower extremities. Cellular immunity is primarily affected and is characterized by mild to moderate lymphopenia, decreased delayed cutaneous hypersensitivity responses, and decreased proliferation in response to phytohemagglutinin (PHA). They are at risk of fatal varicella infections.

Griscelli syndrome: This is an autosomal recessive syndrome of partial albinism, neutropenia and thrombocytopenia, and lymphohistiocytosis. Seizures and neurodegeneration can occur. Melanosomes accumulate in melanocytes, resulting in large clumps of pigment in hair shafts. The absence of giant granules and the histologic characteristics of the hypopigmentation differentiate this condition from Chediak-Higashi syndrome. Most patients suffer from recurrent and severe fungal, viral, and bacterial infections. They have T cell dysfunction, hypogammaglobulinemia, and neutropenia.

Ectodermal Dysplasia: A subgroup of Hypohydrotic ectodermal dysplasia, mainly X linked and some times AR may have associated immune defects, mainly hypogammaglobulinemia or hyper IgM syndrome. They have the typical clinical characteristics of ectodermal dysplasia.

Dyskeratosis Congenita: Dyskeratosis congenita is an X-linked disorder marked by reticulate skin pigmentation, nail dystrophy, leukoplakia of the oral mucosa, aplastic anemia, and an increased risk of malignancy. Progressive bone marrow failure develops in most patients and is the major cause of early mortality. Neutropenia occurs in approximately half of the patients. Both humoral and cellular immune responses may be defective. Thymic aplasia was also reported.

Acrodermatitis enteropathica: This is an autosomal recessive disorder characterized by diarrhea, dermatitis, and alopecia due to inadequate zinc metabolism. Severe infection with opportunistic pathogens occurs frequently and recurrent infection occurs in 30% of patients. Decreased response to PHA and abnormal delayed cutaneous hypersensitivity skin response are typical. Hypogammaglobulinemia and defective chemotaxis of neutrophils and monocytes are variably present. Skin manifestations and risk of infections resolve once the serum Zinc level is normalised.

Netherton syndrome: The triad of trichorrhexis (brittle "bamboo" hair), ichthyosiform erythroderma, and atopic diathesis make up the Netherton syndrome, an autosomal recessive disorder. Recurrent infections, most commonly involving the skin, IgG abnormalities (both hypo- and hyper-IgG), impairment of delayed cutaneous hypersensitivity response, mitogen response, and neutrophil phagocytosis, increased IgE are some of the features.

Leukocyte adhesion deficiency type II (LAD II): This is an autosomal recessive disorder characterized by recurrent infections, persistent leukocytosis, microcephaly, cortical atrophy, short

stature, and severe mental retardation. This condition has also been termed Rambam-Hasharon syndrome and congenital disorder of glycosylation IIc (CDG- IIc). The patient's cells lack fucosylated molecules, including the red blood cell marker H. Deficiency of the erythrocyte H blood group antigen is known as the Bombay blood phenotype.

Although the immune deficiency can be severe in infancy, children that have survived seem to have fewer serious infections and they may have only chronic periodontitis in later childhood. Leukocytosis with neutrophilia is consistently observed. Pus formation is defective, and there is a failure of neutrophil recruitment to sites of inflammation. Neutrophil motility is greatly decreased, although phagocytic activity is normal.

Wiskott-Aldrich syndrome: This well-defined X-linked primary immunodeficiency disorder is characterized by chronic eczema, thrombocytopenia (with small, defective platelets), and bloody diarrhea. Recurrent and life-threatening infections are a leading cause of death. Abnormal humoral immune responses are typical. The disease phenotype ranges from mostly thrombocytopenia to mild or severe forms of the disease. The WAS gene, which is expressed solely in lymphocytic and megakaryocytic lineages, is mutated in Wiskott-Aldrich patients. Inactivating mutations in WAS have also been detected in isolated X-linked thrombocytopenia, while mutations resulting in constitutive activation have been detected in X-linked congenital neutropenia.

Chediak- Higashi syndrome: This well-defined autosomal recessive primary immunodeficiency disorder presents with recurrent bacterial infections (especially with S. aureus and streptococci), partial oculocutaneous albinism, prolonged bleeding time, nystagmus, and neuropathy. Most patients eventually

develop a distinctive lymphoproliferative disorder characterized by generalized lymphohistiocytic infiltrates, which are difficult to treat. The defective gene, CHS1, may code for a protein involved in endosomal trafficking.

Omenn disease: This is an autosomal recessive form of familial histiocytic reticulocytosis that presents with an erythematous skin rash, eosinophilia, reticulosis, hepatosplenomegaly, protracted diarrhea, alopecia, and lymphadenopathy. A characteristic severe combined immunodeficiency leads to failure-to-thrive, recurrent infection, and premature death. Although discussed previously in the context of short-limbed skeletal dysplasia, it usually occurs without associated skeletal anomalies. The immunologic derangements are quite variable and may include abnormal T cell number and function and greatly elevated IgE.

Shwachman Syndrome: This autosomal recessive syndrome presents with pancreatic insufficiency, neutropenia, and metaphyseal dysostosis resulting in short stature. The patients have a predisposition to hematologic malignancy. Neutropenia (which may be intermittent or cyclic) occurs in 88% of patients, and leukopenia and/or pancytopenia may arise.

Pearson syndrome: This mitochondrial disorder features exocrine pancreas dysfunction and bone marrow failure. Mitochondrial DNA deletions have been detected. Surviving patients progress to clinical Kearns-Sayre syndrome, which shows the same mitochondrial DNA changes as in Pearson syndrome.

Glycogen storage disease Ib/Ic: Severe neutropenia, neutrophil dysfunction, defective chemotaxis and microbial killing are associated features.

Galactosemia: A defect in galactose-1-phosphate uridyl transferase results in galactosemia and presents with jaundice, hepatomegaly, cataracts, developmental delay, and feeding difficulties. These patients are at increased risk for fatal sepsis from E. coli in the neonatal period. Impaired chemotaxis is one feature.

IEMs like Methyl Malonic Acidemia, Propionic Acidemia and Iso valeric academia: Can develop sepsis due to neutropenia.

Bloom syndrome: This autosomal recessive condition is characterized by pre- and post-natal growth failure, hypersensitivity to sunlight, and characteristic facial features (malar hypoplasia, micrognathia, and prominent ears). Diabetes mellitus occurs with increased frequency, usually in early adulthood. Risk of neoplasia, especially leukemia and lymphoma, is greatly increased and is the most frequent cause of death. There is an increased susceptibility to infection, especially pneumonia and otitis media. Immunological defects may involve both the humoral and cellular responses, and prolonged low levels of IgM have been reported. Recurrent lung infections can also be there.

Fanconi pancytopenia: This autosomal recessive syndrome is associated with hyperpigmentation of the skin, cafe au lait spots, radial hypoplasia, short stature, microcephaly, renal and genital anomalies, mental retardation and a characteristic facial appearance (microphthalmia, micrognathia, broad nasal base, and epicanthal folds). Single chromatid breaks and gaps, as well as multiradials of the nonhomologous type are present. Increased sensitivity to the clastogenic agent diepoxybutane is useful for diagnosis and prenatal detection, although heterozygotes are not reliably detected. Neutropenia secondary to bone marrow failure occurs in over 95% of patients. T- and B-cell function are generally normal.

Ataxia Telangiectasia: This is an autosomal recessive condition marked by progressive cerebellar ataxia, oculocutaneous telangiectasias, and chromosome instability. Patients with AT are at increased risk for malignancy, especially leukemia and lymphoma. Elevated alpha-fetoprotein is a consistent finding. Most breaks occur at sites involved in the assembly of immunoglobulin and the T cell receptor for antigen (chromosomes 2, 7, 14, 22). There is an increased sensitivity to ionizing radiation. Most patients suffer from clinical immune deficiency, including recurrent sinopulmonary infections, and approximately 10% have a severe immunodeficiency. The severity and type of immune dysfunction are very variable. Low levels of IgA, IgE and IgG are seen.

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Interpretation of CBC and peripheral smear in Primary immune deficiency

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Primary immune deficiencies (PID) are usually first suspected because of infections. A PID might be suspected in children or adults who have infections that are unusually common, severe or persistent, or if they are caused by unusual microorganisms. The types of infections that occur can give a clue to which type of PID is present.

PIDs can also cause the body to attack itself — this is called 'auto-immunity'. This can cause various symptoms, including pain and swelling in the joints ('arthritis'), skin rashes and a loss of red blood cells ('anaemia'). Some PIDs may become symptomatic soon after birth, eg. complete DiGeorge syndrome may result in facial dysmorphism, heart disease and neurological manifestations. A family history of PIDs or compatible symptoms, and complete blood counts, may also help.

When primary immunodeficiency is suspected, initial laboratory studies include a complete blood cell count (CBC) with manual differential, immunoglobulin assays (IgG, IgM, IgA), measurements of circulating antibodies against vaccine antigens, and delayed-type hypersensitivity skin tests. The CBC can detect deficiencies in lymphocytes, neutrophils and platelets. Usually, a

normal CBC eliminates the diagnosis of T-cell defects or combined immunodeficiency.

One should be careful when assessing newborns for immune dysfunction since engrafted maternal T cells, may result in a falsely high lymphocyte count and features of graft-versus-host disease. If severe combined immunodeficiency is strongly suspected and the lymphocyte count is normal , it is necessary to ascertain the origin of the T cells.
When a diagnosis is in doubt, genetic tests or immunophenotyping, may be necessary in consultation with a pediatric immunologist.

Further immunological tests are performed in a stepwise manner to exclude the most severe PIDs upfront, e.g. severe combined immunodeficiency (SCID). These tests include:
• Complete blood count (CBC) with a differential count.
• Measurement of immunoglobulins.

Hematological tests

Complete blood count (CBC)
Several types of blood cells are involved with the immune responses of the body. For PID diagnosis, this should include a 'differential' count of the various white blood cells. The CBC results are compared with the age specific 'reference' values.

The CBC is a crucial, yet under-utilized test and reveals severe defects that could be due to a PID. For example, patients with SCID, one of the most serious forms of PID, typically have very low levels of T cells. SCID is a pediatric emergency as affected infants are at high risk of life-threatening infections. Early diagnosis and therapy are essential to improve outcomes. The

International Patient Organization for Primary Immunodeficiencies (IPOPI) is spearheading efforts to initiate routine neonatal screening for SCID in Europe. Ataxia telangiectasia is associated with a progressive decrease in T cells with time.

The CBC also detects 'neutropenia', that can be a feature of many PIDs (e.g. severe congenital neutropenia and X-linked neutropenia). The CBC can also detect abnormalities of platelet number or size.. Patients with Wiskott-Aldrich syndrome (WAS) have micro platelets and thrombocytopenia and are at risk of hemorrhagic manifestations. The CBC also detects anaemia, which is usually seen in several primary immune deficiencies

These various types of blood cells can be affected by various diseases and drugs, in addition to the different PIDs. The results of the CBC must be interpreted keeping in mind whether any of these factors are present. In addition, as the immune system matures during childhood, the levels of cells must be interpreted according to the age of the child.
The findings on a CBC guides the necessity for more detailed tests, usually performed by a clinical immunologist, if possible.

Other blood cell tests

Lymphocyte subpopulation and proliferation tests: .
T and B lymphocytes can be divided into various subpopulations, e.g. helper T cells or 'CD4' cells and cytotoxic T or CD8 cells. Enumeration of basic lymphocyte subsets can identify which PID is present. In addition to enumeration, it is also important to test functionality. For example, certain tests identify how well these cells proliferate when they are stimulated.

Granulocyte function tests:
Neutrophils, eosinophils, basophils and mast cells are together known as 'granulocytes'. Normally these cells (mainly neutrophils) produce hydrogen peroxide (sometimes called 'reactive oxygen') to kill bacteria and fungi. The amount of hydrogen peroxide produced is usually measured using a laboratory test called the dihydrorhodamine (DHR) oxidative burst test. This is an important diagnostic test for the PID known as X-linked chronic granulomatous disease (CGD), in which the neutrophil count is normal (or high), but these cells do not work properly. Other tests are used to assess how well these cells migrate toward an attractant (this is called 'chemotaxis') and how effectively they kill and swallow bacteria.

B cell maturation tests:
This test is used to diagnose agammaglobulinemia, such as X-linked agammaglobulinemia (XLA, also known as Bruton disease) caused by genetic defects that prevent normal maturation of B lymphocytes. Normally, mature B lymphocytes, ie. plasma cells produce immunoglobulins. Patients with these disorders therefore have very low levels of immunoglobulins as well as low B cell numbers.
Cell protein expression:
Tests can identify deficiencies in proteins usually present on the surface of leukocytes. For example, CD40 and CD40L allow T helper cells to stimulate B cells. CD40 or CD40L deficiencies are severe PIDs also known as hyper IgM syndrome. Defects in CD11 and CD18 expression can cause leukocyte adhesion deficiency (LAD).

Switched memory B cells:
Memory B cells can 'remember' antigens that the body has previously encountered. When stimulated again, they 'switch' on

to produce the required antibodies. Enumeration of switched memory B cells can be useful in diagnosis of common variable immunodeficiency (CVID), hyper-IgE syndrome, and CD40/CD40L defects.

A complete blood count and a peripheral smear remain the initial tests of choice in all suspected PIDs.

Role of Flow cytometry in the diagnosis of primary immunodeficiency disorders

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Primary immunodeficiency disorders (PIDs) are rare inherited disorders comprising of more than 350 different genetic diseases of the immune system. The common clinical manifestations are repeated infections, allergies, autoimmune diseases, and in some cases, malignancies.

During the last two decades, flow cytometry has become a rapid, sensitive and reproducible diagnostic tool for PIDs. The machine provides phenotypic characterization of a cell type using monoclonal antibodies. The initial flow cytometry results, together with the available clinical data helps in making a decision on further testing, especially genetic testing.

Evaluation of 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, and it is further sub classified based on the B cells and NK cells count in these patients. With flow cytometry, expression of CD132 and CD127; functional assays like pSTAT5 activation on

T lymphocytes after IL-2 stimulation can be detected which is essential for specific diagnosis of various CID.
Patients with Omenn syndrome, MHC-I or MHC-II deficiency, ZAP 70 deficiency, etc can have normal T cell numbers. 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) by flow cytometry helps in patient evaluation in such cases.

In our experience we have observed that T naïve cells are helpful to differentiate SCID patients from transient lymphopenia caused due to recurrent infections. Children with SCID show lack of naïve cells (CD45RA+/CD62L+ T cells) or may have CD45RO+ T cells (memory phenotype) whereas in children with transient lymphopenia the expression of naïve cells are normal.

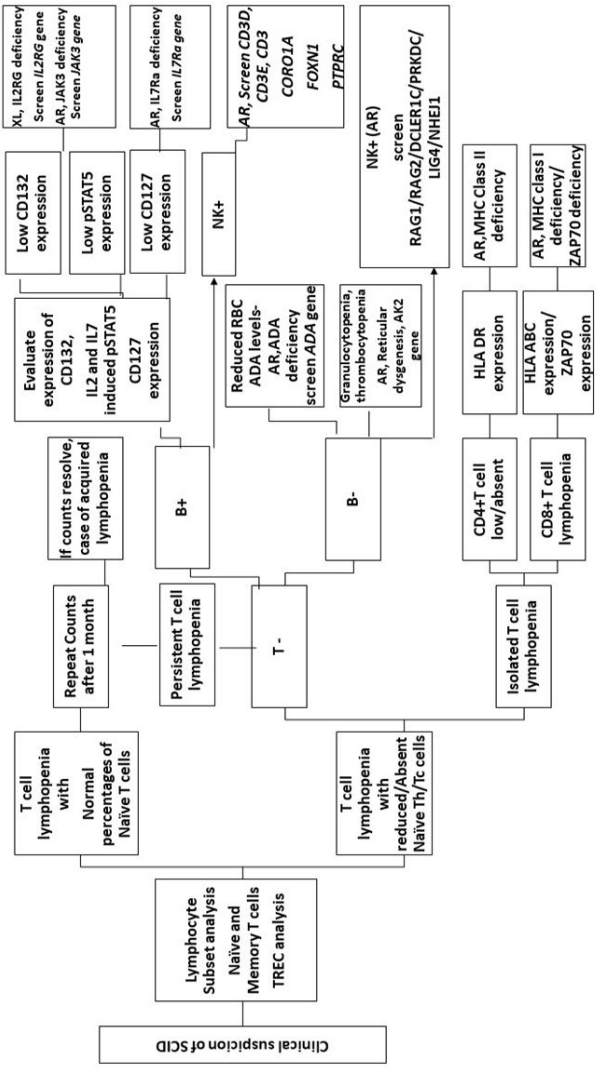

Figure 1: An algorithm for laboratory evaluation of patients with combined T & B cell defects

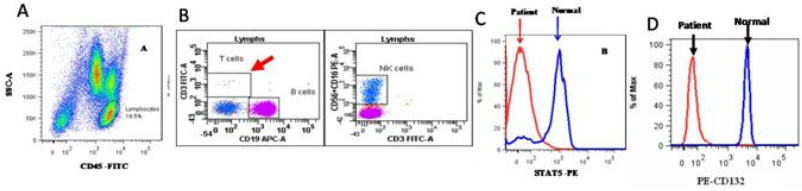

Figure 2: Flow cytometry work up in a patient with X-linked severe combined immunodeficiency. Dot plot (A) showing gating of lymphocytes using CD45 gating strategy. Dot plot (B) showing T-B+NK+ SCID. Histogram (C) showing abnormal expression of pStat5 in patient compared to normal. Histogram (D) showing abnormal expression of CD132 in patient compared to normal.

Evaluation of CID with associated or syndromic features:
Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by the triad of thrombocytopenia, eczema, and combined B and T-cell deficiency. This disease is caused by a defect in the WAS protein gene. In WAS the expression of this glycoproteins is decreased which is normally expressed on all lymphocytes, neutrophils, macrophages, and platelets. WAS patients are diagnosed by analyzing expression of intracellular WAS protein which shows absence or reduced expression in lymphocytes and myeloid cells.

Evaluation of patients with 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 can be X-linked (XLA) or autosomal recessive agammaglobulinemia. On the other hand, reduced immunoglobulin levels with low or normal B cells are suggestive of

common variable immunodeficiency (CVID). Low IgG and IgA with normal to high IgM levels are indicative of Hyper IgM (HIGM) syndrome. These defects can be evaluated by determining the expression of CD40 and CD40L (CD154) on B and T cells respectively. Patients with X-linked HIGM will have abnormal CD 154 expression on T cells after stimulation.

Patients with these disorders may however, have normal or modest reduction in immunoglobulin levels. Thus, measurement of serum antibody titer (specifically IgG) in response to vaccine antigens can be the best method to confirm the diagnosis of an antibody deficiency disorder.

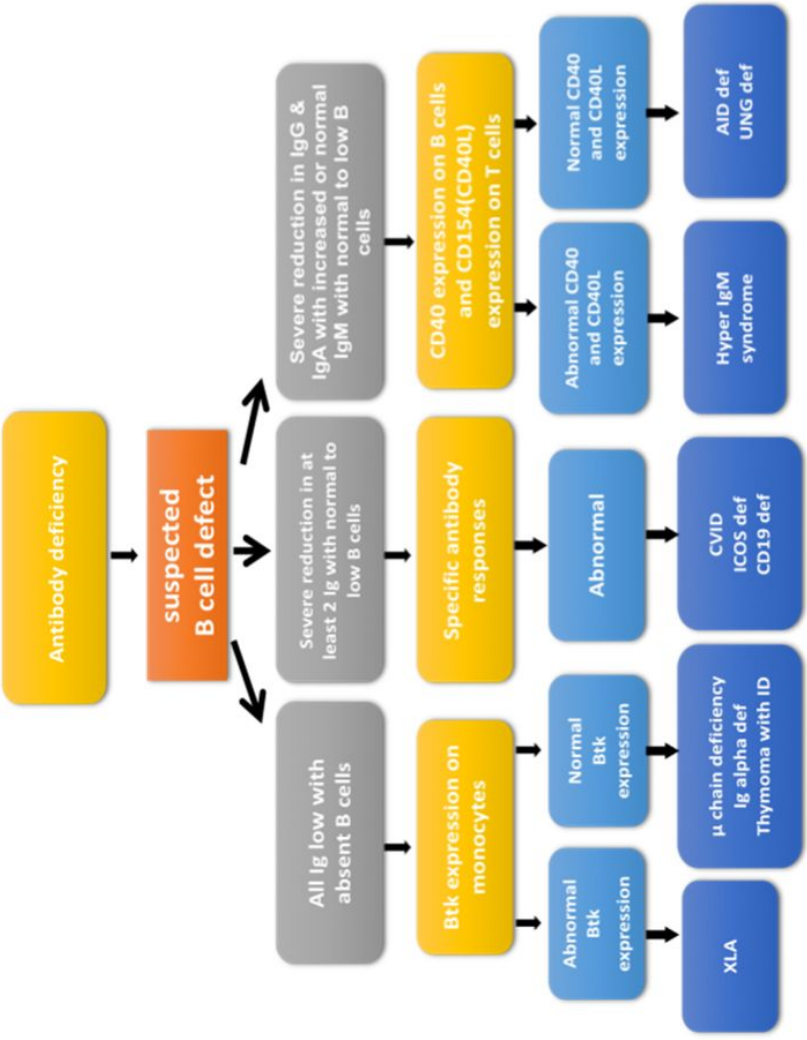

Figure 3: Diagnostic approach for B cell defects.

Evaluation of patients with 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 two to four fold of normal count. Bone marrow examination shows the presence of early precursor cells but very few mature cells beyond the promyelocyte stage (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 0.2x109/l for 3-5 days, after which they rise rapidly to levels near the lower limit of normal, about 2x109/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.

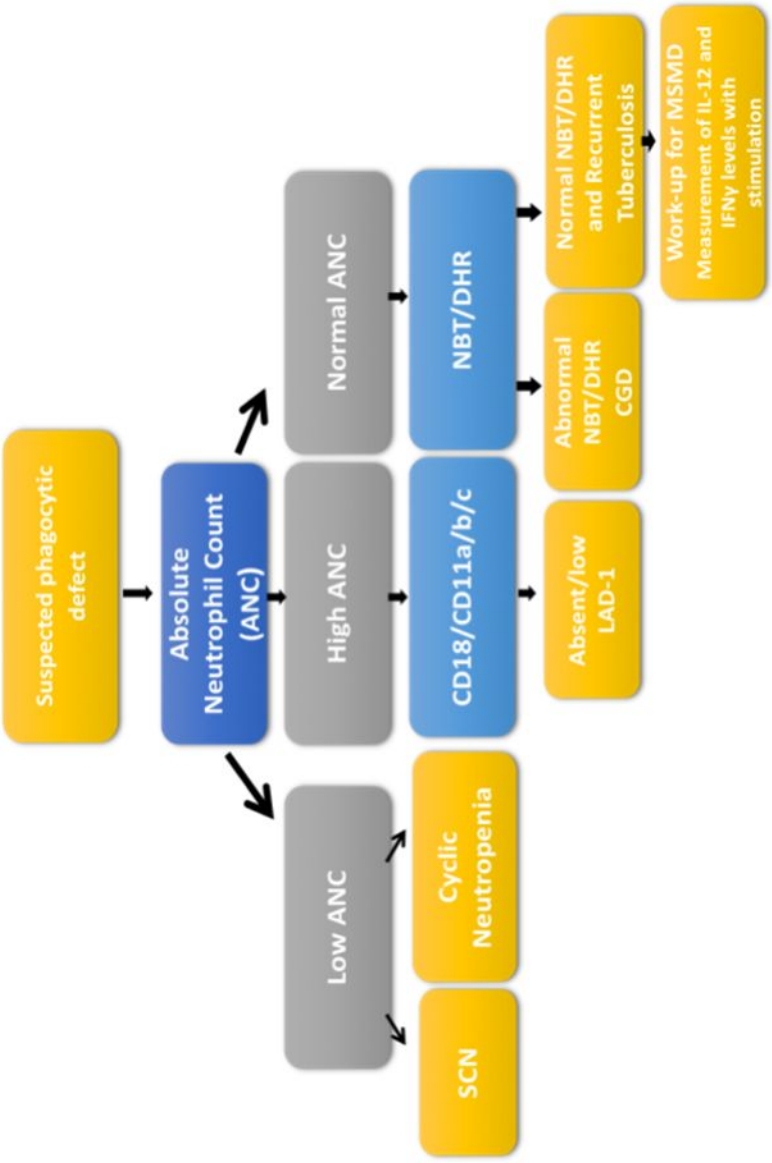

Figure 4: An algorithm for evaluation of Patients with phagocytic defects.

Leukocyte Adhesion Deficiencies

Leukocyte Adhesion Deficiency-1: Leukocyte adhesion deficiency type 1 (LAD-1) is a rare, inherited immunodeficiency with defect in the recruitment of leukocytes to the site of inflammation. It results from mutations in the ITGB2 gene encoding for the β2 subunit (CD18) of beta 2-integrin. Patients with severe LAD-I have absent or markedly reduced expression of CD18 and CD11.

Leukocyte Adhesion Deficiency-2: LAD type II syndrome is an extremely rare form of adhesion defect which results from a defect in fucose metabolism, causing the absence of SLeX (CD15s) a carbohydrate ligand on the surface of neutrophils.

Chronic Granulomatous Disease (CGD) workup:

CGD is a heterogeneous group of inherited disorders characterized by a defect in any one of the 5 components of nicotinamide adenine dinucleotide phosphates (NADPH) oxidase subunits, required for phagocytic killing. Previously CGD was mainly diagnosed by Nitroblue tetrazolium test (NBT) which is now replaced by flow assay which measures the neutrophils' ability to generate an oxidative burst using fluorescence dye such as Dihydrorhodamine 123 (DHR). In DHR test, Phorbol 12-myristate 13-acetate (PMA) acts as a stimulant and Dihydrorhodamine 123. In presence of reactive oxygen intermediates generated during the respiratory burst, the dye is rapidly oxidized to produce the brightly fluorescent cationic compound Rhodamine 123, which localizes in the mitochondria. This is evaluated using flow cytometer which determines the percentage of phagocytic cells producing reactive oxidants (conversion of DHR 123 to R 123). Expression of DHR on neutrophils in affected individual is less than 1% and in carrier the two distinct subsets of neutrophils appear.

Evaluation of patients with intrinsic and innate immunity defects
Mendelian susceptibility to mycobacterial diseases (MSMD) is a rare form of primary immunodeficiency. Patients with MSMD have increased susceptibility to systemic infections like salmonellosis, tuberculosis, and severe infection with weakly virulent non-tuberculous mycobacteria (NTM) including the Bacillus Calmette-Guérin (BCG) vaccine strain. To date the genetic defects for MSMD have been identified in members of the interleukin-12 (IL-12) –IL-23–interferon-γ (IFNγ) signaling pathway. MSMD work up involves flow cytometric evaluation of CD119 and IFNγR2 expressions on monocytes for differential diagnosis of IFNγR1 and IFNγR2 deficiency respectively.CD212 expression on activated T cells is performed to rule out IL-12Rβ1 deficiency. The functional assays like pSTAT1 are performed to rule out IFNγR1 and IFNγR2 deficiencies and pSTAT4 assay is done for evaluation of IL-12Rβ1 deficiency.

Evaluation of Patients with Immune Dysregulation
Familial Hemophagocytic Lymphohistiocytosis (HLH): HLH is a life-threatening condition resulting from impaired NK cell function clinically characterized by fever, splenomegaly, cytopenias and hemophagocytosis. It is important to differentiate HLH from severe infection and septicemia, as the management in both of them differs significantly. Primary HLH occurs due to inherited genetic defect leading to impaired NK cell function while Secondary HLH is often associated with infections, malignancies, rheumatic diseases. Macrophage-activation syndrome (MAS) is a severe, life threatening complication of several chronic rheumatic diseases of childhood which has clinical and laboratory features of HLH. Further evaluation using NK cell cytotoxicity assay, Perforin, SAP, XIAP expression studies and Granule release assay on lymphocytes by flow cytometry and

MUNC 13-4 and SYNTAXIN-11 by western blot helps significantly in diagnosis of genetic HLH.

Autoimmune lymphoproliferative syndrome (ALPS):
It is a defect of FAS-mediated apoptosis pathway mainly characterized by non-malignant lymphoproliferation of blood cells and increased risk of lymphoma. Cardinal clinical features of ALPS include hepatosplenomegaly, lymphadenopathy and autoimmunity. Initial laboratory evaluation includes flow cytometric analysis of peripheral blood circulating TCR αβ+ DNT cells and estimation of serum Vitamin B12, soluble FAS ligand (sFASL), interleukin IL-10 and IL-18 levels. The presence of elevated TCRαβ+ DNT cells (≥1.5% of total lymphocytes or ≥ 2.5% of T 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.

Evaluation of complement deficiency, auto-inflammatory disorders phenocopies of PID:
Flow cytometry application is limited in complement deficiency and auto-inflammatory disorders. However, some of the phenocopies of PID can be evaluated using flow cytometry by identifying auto antibodies against specific cytokines and by looking into specific pathways.

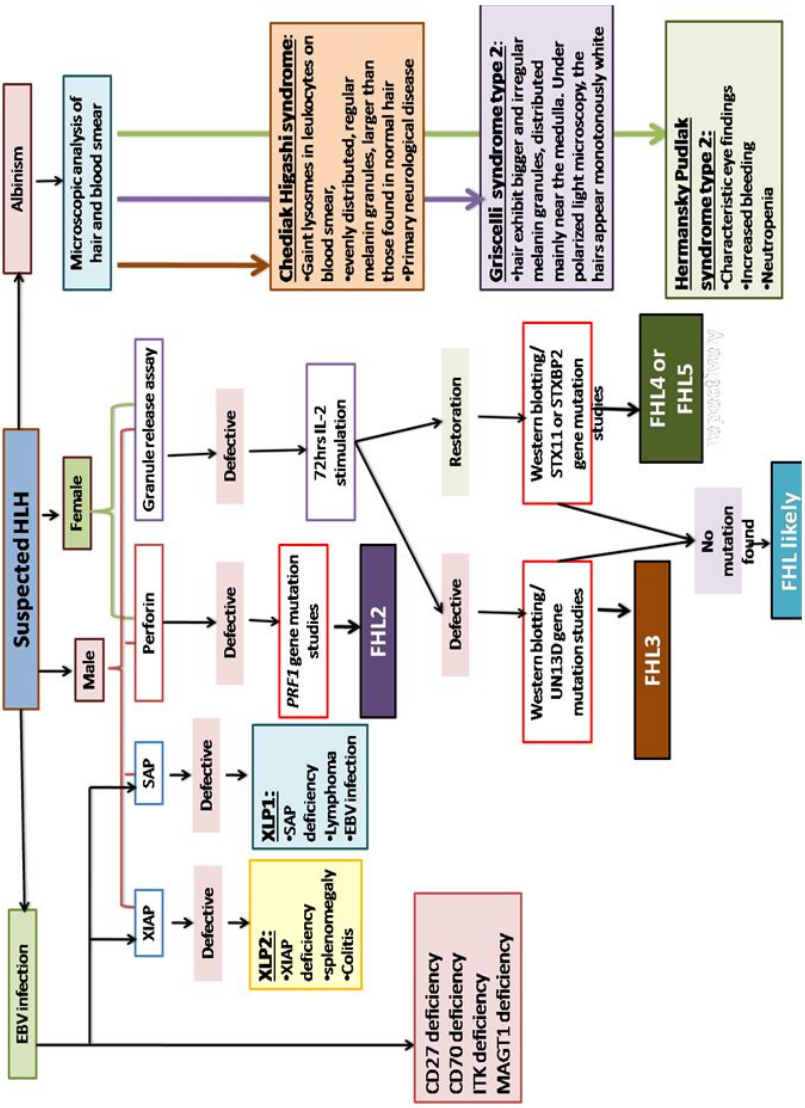

Figure 5: Diagnostic approach of Hemophagocytic Lymphohistiocytosis (HLH)

Conclusion

Flow cytometry helps in a comprehensive evaluation of the immune system in patients with suspected PID. In certain situation analysis of specific protein expression helps in diagnosis of specific PID. However, one has to remember that normal expression does not rule out abnormal function and hence further analysis in the form of functional assays or molecular testing is required in cases with strong clinical evaluation. Flow cytometry also helps in functional validation of the mutations identified either by Sanger sequencing or by NGS analysis.

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Oxidative burst assays

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Oxidative burst or respiratory burst is a normal response from phagocytic cells on activation by stimuli, especially microbes. It is the enzymatic generation of reactive oxygen species from molecular oxygen, critically involved in microbial killing. At least 5 clinically significant disorders of oxidative pathways are known involving -

1.NADPH oxidase
2.Glucose 6 Phosphate Dehydrogenase
3.Myeloperoxidase
4.Glutathione reductase
5.Glutathione synthetase

Of these, NADPH oxidase and its most important disease association, namely Chronic granulomatous disease are the focus of interest here.

NADPH oxidase is a complex multiunit molecule. It is a flavine adenine dinucleotide (FAD) dependent electron transferase located on the plasma membrane, phagolysosomal membrane as well as in cytoplasm which lie separate and assemble only on activation by stimuli such as microbes. Then it shuttles electrons across the membrane to molecular oxygen, generating superoxide,

hydrogen peroxide and hydroxyl radicals and hypochlorite ions with the help of myeloperoxidase.

The subunits of the NADPH oxidase are individually designated with a suffix phox for phagocyte oxidase. They are $gp91^{phox}$ and $p22^{phox}$ on the membrane and, $p47^{phox}$, $p67^{phox}$ and $p40^{phox}$ in the cytoplasm. Mutation of any of the subunits can result in Chronic granulomatous disease. Nevertheless, more than two thirds of cases are due to deficiency of $gp91^{phox}$ which is X linked. All others are autosomal recessive in inheritance. There is also a GTP binding Rac protein associating with the complex, but no disease association is documented by its deficiency.

Chronic granulomatous disease is a primary immunodeficiency disease manifesting in early childhood with recurrent severe infections caused by bacteria or fungi in multiple organs, with formation of granulomas and micro abscesses. Although rare, high index of suspicion may help picking up cases who can be treated with high success rate.

Diagnostic investigations mainly include:

1.Nitro blue tetrazolium dye test:
Blood sample is incubated on a slide coated with Phorbol ester or lipopolysaccharide. Alternatively, it can be done in glass tubes. Activated neutrophils and monocytes convert colourless NBT to blue formazan.

NBT reduction (absence of cells with dark blue formazan deposits)is absent in both X-linked and AR forms of CGD (Fig. 1).

• In X-linked carrier state approximately 50% of neutrophils fail to reduce NBT. The percent positive cells can vary if there is unequal X inactivation and may appear normal or like CGD with extreme lyonization.

• False-positive results can occur (i.e., apparent failure to reduce NBT supporting the diagnosis of CGD) if the neutrophils do not adhere to the slide. This happens with greasy slides or with some cases of LAD. Using phorbol myristate acetate to stimulate the cells will avoid this.

2.Dihydroxyrhodamine (DHR) test by flow cytometry

Dihydroxyrhodamine is converted to brightly fluorescent rhodamine by stimulated phagocytes. The degree of fluorescence gives a quantitative assessment of the oxidative burst.

• This approach has replaced the NBT slide test in many laboratories. It has the advantage of assessing large numbers of cells and can give quantitation of the amount of oxidant production.
• X-linked CGD patients will not respond at all and show no increase in fluorescence with stimulation
• X-linked carriers will show about 50% of the cells that respond with a normal increase in fluorescence, and the other half will have no response. Degrees of unequal X inactivation are much more accurately quantified by this assay
• AR patients, particularly those with absent p47phox, have some response to stimulation and show a small increase in fluorescence. This level of oxidant production is usually not visible on the NBT test.
• AR carriers have a good response, but the histogram may be broader than normal and may even appear bimodal with a weakly

fluorescent peak and a strongly fluorescent peak. This is not distinguishable on the NBT slide test.

• Falsely negative results not supporting the diagnosis of CGD have been reported in specimens that have been run a few days after phlebotomy.

• Falsely abnormal results suggesting CGD can be seen in patients with MPO deficiency because MPO is required to generate strong DHR fluorescence

3. BURST TEST

Rapid and sensitive method for diagnosis of CGD and for detection of X linked carriers. It utilizes a kit containing opsonized bacteria, PMA (Phorbol 12.Myristate 13.Acetate) and the chemotactic peptide N.formyl Met-Leu-Phe(fMLP) as stimulants, DHR as a fluorogenic substrate and other reagents. It allows quantitative determination of leucocyte oxidative burst in heparinized whole blood. It is also useful for the evaluation of effect of immunomodulator drugs like GM-CSF, G-CSF etc.

Primary immunodeficiency disorders with suspected Autoimmune/ Inflammatory manifestations

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Primary immunodeficiency disorders (PIDs), autoimmune diseases and autoinflammatory diseases, allergy and malignancy are different facets of a dysfunctional immune system. PIDs comprise a heterogeneous group of inherited disorders affecting one or multiple components of the immune system and characterised by an increased susceptibility to infections. PIDs can result from defects of adaptive immune system (T-cell and B-cell) or innate immune system (phagocyte and complement disorders). At the other end of the spectrum, dysregulated activation of adaptive and innate immune system gives rise to autoimmune diseases and autoinflammatory diseases, respectively. Less of immunity causes immunodeficiency and more causes autoimmunity/autoinflammation is an oversimplified and a flawed concept. This notion fuelled the belief that concurrence of immunodeficiency and autoimmune/autoinflammatory is paradoxical. Over the years, many scenarios of such overlap have been reported and studied. Almost the entire spectrum of autoimmune diseases have been reported across PIDs. Unravelling of shared genetic mutations and immune pathways has given a

better insight into the complexity of the functioning of the immune system. Co-existence of immunodeficiency and autoimmunity is now considered logical. In this review, we shall cover the following aspects- mechanisms of autoimmunity/inflammation in PIDs, types of autoimmune/inflammatory manifestations in PIDs, evaluating and managing these manifestations.

Mechanisms of autoimmunity/inflammation in PIDs

A robust immune system differentiates self antigens from foreign, and mounts a regulated immune response against a non-self antigen while at the same time, exhibits tolerance to self antigens. Functions of immune response and tolerance are interconnected. With this central idea, the following explanations have been put forth to substantiate autoimmunity in PIDs.

First, a genetic defect in immune system not only impacts the ability of immune system to fight against infections but can also affect mechanisms of tolerance. T and B cells acquire central tolerance in the thymus and bone marrow, respectively (1). At these sites, high affinity self reactive lymphocytes are deleted. If there is a defect that prevents deletion of these "rogue" lymphocytes, this results in autoimmunity. Few "rogue lymphocytes" can escape from the thymus into the periphery even in normal instances. However, their action is neutralised by presence of T regulatory cells and this constitutes an important mechanism of peripheral tolerance. Defective T regulatory cells also results in autoimmunity. Defective central and peripheral tolerance is exemplified by APECED and IPEX, respectively where autoimmunity is seen in 100% of cases (2).

Second, a patient with PID is unable to clear infections effectively; thereby resulting in exposure of a dysfunctional immune system to

multiple chronic infections. These chronic infections stimulate the immune system via mechanisms of molecular mimicry, by-stander activation and superantigen stimulation. Also, compensation for an ineffective system often results in chronic, exaggerated, dysregulated inflammation causing damage to the host tissue (1).

Third, large quantity of apoptotic debris is generated as a consequence of chronic infection and dysregulated inflammation. Further, defect in complement pathway or defective phagocytosis prevents clearance of apoptotic debris. These debris further causes immune stimulation (1,2).

Manifestations of Autoimmunity in PIDs

Next to infections, autoimmune or inflammatory features are the most common clinical manifestations of PIDs.(3) In some instances, features of autoimmunity can precede infections in PIDs. Hence, it is advocated that autoimmunity should be included in the warning signs of primary immune deficiency. Younger age at onset of autoimmunity and multiorgan autoimmunity that does not fit into the label of a single rheumatological illness should raise suspicion of PIDs. Further, non-infectious complications contribute significantly to morbidity in PIDs. The largest data on prevalence of autoimmune and inflammatory disease in PID comes from the French CEREDIH PID registry (4). Autoimmune and inflammatory disease was seen in 26.2% of patients and these features had negative prognosis overall and worse outcome after Haematopoietic Stem Cell Transplant (HSCT). Notably, risk was higher in children but no gender bias was seen. T-cell PIDs and common variable Immunodeficiency were associated with the highest risk. The most commonly reported features were autoimmune cytopenias, inflammatory bowel disease and arthritis. Risk of autoimmune cytopenia in PIDs was 120 times that of the

general population. In children with PIDs, risk of IBD was 80 times higher as compared to age matched healthy children (4). High prevalence of autoimmunity was also reported in 247 patients with PIDs in the Slovenian National Registry (5). The prevalence of non-infectious manifestations was as follows: autoimmune manifestations in 22 %, lymphoproliferative/granulomatous in 12 %, autoinflammatory in 5 % and allergic manifestations in 4 %. Autoimmunity was the presenting manifestation of PIDs in 17.8%. This data underlines the need to evaluate for autoimmunity in all patients, especially children with PIDs and vice-versa.

Brief Description of few PIDs and associated Autoimmunity

The prevalence and nature of autoimmune/inflammatory diseases depends on the type of underlying PIDs as depicted in Table 1. As a category, diseases of immune dysregulation had the highest incidence of autoimmunity. Disorders of immune dysregulation include diseases affecting genes affecting central and peripheral tolerance (6). Autoimmunity is the defining feature of these diseases and is seen in 100% of cases. Breakdown of central tolerance in T cells is exemplified by APECED (autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy) resulting from AIRE mutation. APECED is characterised by organ specific and autoimmunity especially involving endocrine organs. Characteristic features involve chronic mucocutaneous candidiasis occurring by age 5 (in 75%) and followed by autoimmune hypoparathyroidism and autoimmune adrenocortical insufficiency occurring by the age of 10 (in 89%) and age 15 (in 60%), respectively (6). Characteristic example of loss of peripheral tolerance is seen in Immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome (2,6).This disorder arises due to mutation in FOXP3 which is the transcription factor for T regulatory cells. The syndrome is characterized by neonatal onset

severe enteropathy, early onset type I diabetes mellitus (T1DM), hypoparathyroidism, thyroiditis, chronic dermatitis, other autoimmune features such as cytopenias, nephritis and hepatitis. Eosinophilia and increased IgE levels are notable features of IPEX. Further, IPEX like syndromes have also been described with mutations in other genes such as CD25, STAT5B, ITCH, STAT1, and STAT3 (7). Treg cells functions are also impaired in lipopolysaccharide responsive beige-like anchor protein (LRBA) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) deficiency (6). LRBA and CTLA4 deficiency develop diffuse lymphocytic aggregates in gut, lungs, brain, and other organs. Consequently, these diseases are characterised by enteropathy, granulomatous lymphocytic interstitial lung disease (GLILD), autoimmune cytopenias and thyroiditis. In addition, CTLA4 deficiency is also unique for the presence of hypogammaglobulinemia(6). Hemophagocytic Lymphohistiocytosis(HLH) and Autoimmune lymphoproliferative syndrome(ALPS) are also categorised as disorders of immune dysregulation. Impaired FAS-mediated apoptosis is the cause for ALPS. Patients present with lymphadenopathy, hepatosplenomegaly and have high risk for lymphoma. Autoimmune cytopenia, is seen in over 70% of patients. Other autoimmune complications include autoimmune hepatitis, Guillain-Barré syndrome, SLE, glomerulonephritis, and uveitis(6).

Omenn syndrome is an example of SCID where autoimmunity is a characteristic feature. Hypomorphic mutations in recombination-activating gene (RAG) 1 or RAG2 are seen. Failure of central tolerance of T cells and defective function of T reg contribute to the development of autoimmunity. T cells infiltrate multiple organs and patients present with enteropathy, early onset erythroderma, alopecia, lymphadenopathy ,hepatosplenomegaly,

and failure to thrive. Severe hypogammaglobulinemia with increased levels of IgE are seen (2,6).

Hyper-IgM syndrome is a heterogeneous condition. Prevalence of autoimmunity depends on the genes mutated, and is as follows- activation-induced cytidine deaminase(AID) (25%), NF-κB essential modulator (NEMO) (23%), and CD40L (20%). Arthritis, cytopenias, IBD, autoimmune hepatitis, endocrine disorders and uveitis have been reported(8).

In CVID, autoimmune diseases are present in 20% (2) . Autoimmune thrombocytopenia followed by autoimmune hemolytic anemia were the most common.Other autoimmune diseases reported include pernicious anemia, SLE, JIA , T1D ,vitiligo, and IBD. Between 7-36% of patients with IgA deficiency had features of autoimmunity (9). Both systemic and organ specific autoimmunity have been described including RA, lupus, celiac disease, autoimmune thyroiditis, autoimmune cytopenia, T1DM, vitiligo, and psoriasis have been reported. In X linked agammaglobulinemia, arthritis,cytopenias insulin-dependent diabetes mellitus,dermatomyositis and scleroderma have been reported(10).

Wiskott–Aldrich syndrome (WAS) presents with recurrent bacterial infections, eczema, and thrombocytopenia with small platelets .WAS is associated with high prevalence of autoimmunity in 70% of cases (11). Autoimmune haemolytic anaemia (AIHA) is the most common manifestation. Other autoimmune features include arthritis, vasculitis, neutropenia, inflammatory bowel disease and IgA nephropathy.

After disorders of immunoregulation, the category of PIDs with highest risk of autoimmunity is early complement deficiency. SLE

is found in 90% of patients with C1q deficiency and in other classical complement (C1r, C1s, C2 and C4) though the risk is lesser as compared to C1q(2). SLE occuring in C1q deficiency is associated with an early-onset, extensive rash, photosensitivity and oral ulcers.

Patients with chronic granulomatous disease (CGD) have a defective production of reactive oxygen species resulting in impaired phagocytosis. Resultant impaired antigen clearance, impaired phagocytosis of apoptotic bodies drives intense inflammation and granuloma formation. In a French cohort, inflammatory manifestations were reported in 69.4% of patients (12). Notably, the risk of inflammatory episodes was twice as much higher in patients with XL-CGD than in patients with AR-CGD. Granulomatous inflammation in gastrointestinal tract and urogenital tract resulted in obstructive features. In addition, granulomas were also found in liver, spleen, lung and brain. Further, IBD-like symptoms were also seen with gastrointestinal involvement. In lungs, granulomatous and interstitial lung disease was reported. Moreover, systemic autoimmune manifestations are found in 10% of CGD and include systemic lupus erythematosus, idiopathic thrombocytopenic purpura,RA,ITP,T1D and juvenile idiopathic arthritis(2).

Table 1 PID and Autoimmunity

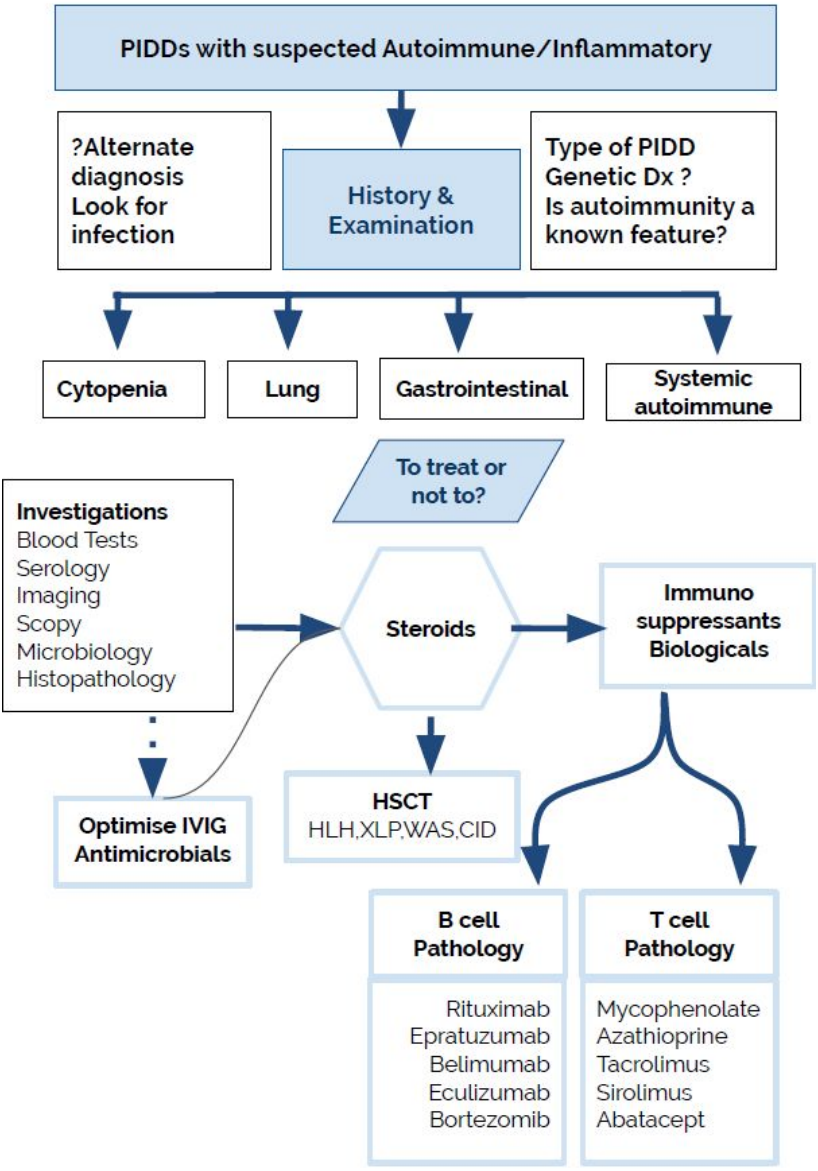
PID	**IUIS Classification category**	**Gene**	**Cytopenia**	**Gut**	**Lung**	**Skin**	**Systemic**	**Others**
Omenn syndrome	Category I Immunodeficienci	RAG1 / RAG2	AIHA, ITP, AN	IBD		Erythroderma		

	es affecting cellular and humoral immunity					Alopecia		
AR Hyper IgE syndrome		DOCK8	AIHA	Enteropathy			Vasculitis	Thyroiditis
Hyper IgM syndrome		CD40LG CD40	AIHA, AN, ITP	IBD			Arthritis	Biliary tract/liver disease, thyroiditis Sclerosing cholangitis
Wiskott-Aldrich syndrome	Category II CID with associated or syndromic features	WAS	ITP AIHA AN Evans	IBD		Eczema	Vasculitis Arthritis Dermatomyositis	IgA nephropathy Uveitis
Hyper IgM syndrome		NEMO	AIHA ITP	IBD				Hepatitis
DiGeorge syndrome		TBX1	ITP AIHA Evans			Psoriasis Vitiligo	Vasculitis Arthritis	Thyroiditis
Ataxia telangiectasia		ATM	AIHA ITP			Alopecia	Vasculitis	Thyroiditis
X-Linked Agammaglobulinemia	Category III Predominantly Antibody	BTK	ITP AIHA	IBD-like disease			RA/JIA	T1DM, thyroiditis

CVID	deficiencies	Polygenic	ITP AIHA AN Evans	AIE IBD Atrophic gastritis Celiac-like disease	GLILD	Alopecia Vitiligo Psoriasis	RA Vasculitis SLE Sjogren's	T1DM, Thyroiditis Pernicious anemia Granulomatous liver disease Hepatitis Nodular regenerative hyperplasia
Hyper IgM Syndromes		AID	AIHA, ITP, IBD	IBD			polyarthritis	Type I DM Hepatitis chronic uveitis
Selective IgA deficiency			AIHA ITP	Celiac disease IBD		Vitiligo	SLE JIA	T1DM, thyroiditis
APECED	Category IV Diseases of Immune Dysregulation	AIRE		Celiac disease IBD	ILD	Vitiligo Alopecia Ezcema	Sjogren's	Hypoparathyroidism Hypothyroidism Adrenal insufficiency T1DM Gonadal dysfunction Pernicious anemia Hepatitis Primary biliary cirrhosis

IPEX		FOXP3	AIHA ITP AN	AIE		Dermatitis Vitiligo Alopecia Psoriasis	Arthritis	Early onset T1DM, Thyroiditis Hepatitis
CTLA4 deficiency		CTLA4	AIHA ITP AN	AIE	LIP GLILD	Psoriasis Vitiligo	Arthritis	T1DM, thyroiditis
LRBA deficiency		LRBA	ITP AIHA AN	AIE	GLILD	Ezcema Alopecia	Arthritis	T1DM, thyroiditis, hepatitis, Uveitis
STAT3 GOF mutation		STAT3	ITP AIHA AN Evans	AIE	GLILD	Ezcema Alopecia	Arthritis	T1DM, thyroiditis, hepatitis
ALPS		TNFRSF6 TNFSF6	ITP AIHA AN Evans			Dermatitis	Arthritis cutaneous vasculitis	Glomerulonephritis optic neuritis,uveitis Guillain-Barre syndrome primary biliary cirrhosis Autoimmune hepatitis

HLH		Poly genic	Cytope nia					Severe inflammat ory syndrome Persistent fever Hepatospl enomegal y Liver dysfuncti on CNS inflammat ion
CGD	Category V Congenit al defects of phagocyt e	CYB B NCF 1 CYB A NCF 4 NCF 2	ITP	IBD		DLE	SLE RA JIA	Lymphop roliferativ e pathology with severe multiorga n granulom atous disease (GI tract, lungs, kidneys, eyes) Chorioret initis Uveitis
C1q,c1r ,c1s,C4, C2 deficien cy	Category VIII Comple ment deficienci es	C1q, c1r,c 1s,C 4,c2						SLE hereditary angioede ma


PIDDs with suspected Autoimmune/Inflammatory
?Alternate diagnosis Look for infection
History & Examination
Type of PIDD Genetic Dx ? Is autoimmunity a known feature?
Cytopenia
Lung
Gastrointestinal
Systemic autoimmune
To treat or not to?
Investigations
Blood Tests
Serology
Imaging
Scopy
Microbiology
Histopathology
Steroids
Immuno suppressants Biologicals
Optimise IVIG Antimicrobials
HSCT
HLH,XLP,WAS,CID
B cell Pathology
Rituximab
Epratuzumab
Belimumab
Eculizumab
Bortezomib
T cell Pathology
Mycophenolate
Azathioprine
Tacrolimus
Sirolimus
Abatacept

PIDs and Autoinflammatory Diseases

Autoinflammatory diseases are the seventh category of nine categories of PIDs classified by the International Union of Immunological Societies. For the purpose of this review, only autoinflammatory with "immunodeficiency" or recurrent infections as a characteristic feature shall be mentioned. With the advent of next generation sequencing, monogenic autoinflammatory diseases with immunodeficiency features have been described. Detailed description of these conditions is outside the scope of the discussion.These include DADA2 (Deficiency of Adenosine deaminase 2), PLCG2-associated antibody deficiency and immune dysregulation (PLAID), APLAID, Linear ubiquitination assembly complex (LUBAC) deficiency, Periodic fever, immunodeficiency and thrombocytopenia (PFIT) and Sideroblastic anemia with immunodeficiency, fevers and developmental delay (SIFD) have been described in recent years. DADA2 is characterised by fever, livedoid racemosa, lacunar infarct, polyarteritis nodosa, hepatosplenomegaly .PLAID and APLAID have characteristic cutaneous features- cold induced urticaria and blistering skin lesions, respectively. Other features seen in APLAID are early onset bloody diarrhea, NSIP, arthralgias and uveitis.These conditions are characterised by recurrent bacterial and viral infections secondary to antibody deficiency (13).

Evaluating and managing autoimmune manifestations in PIDs

Personalised and vigilant approach is required while evaluating for a possible autoimmune manifestation in a patient with PIDs and this approach has been outlined in Figure1. The following scenarios pose unique challenges for the treating clinician in managing autoimmunity in PIDs. The biggest hurdle is differentiating between inflammation/autoimmune and infection.

In India, methods for investigating for infections with the existing resources are far from ideal, even in an immunocompetent individual. In a patient with defective immune system, there is always a possibility of infection by a typical/ atypical organism triggering a state of hyperinflammation or mimicking autoimmune features. Meticulous history and clinical evaluation complemented by lab tests, radiological investigation and histopathology are required to confirm diagnosis.

Alternate diagnosis should always be considered for every scenario suspected to be due to autoimmunity. This is necessary especially as there are no absolutely confirmatory tests for most of the autoimmune manifestations and antibody tests such as ANA positive or DCT positive can be seen even in a patient without autoimmune features. Further, the utility of autoantibody testing may be impaired in PIDs with hypogammaglobulinemia. Monthly IVIG therapy can also interfere with serologic testing; hence it has been suggested to use stored sera drawn prior to therapy for autoantibody assays (3). While performing radiological investigations, it should be borne in mind that some PIDs such as ataxia telangiectasia are radiosensitive and hence exposure should be limited.

Equally challenging is the prospect of administering immunosuppressive treatment and risk of added infectious risk in a patient with already defective immune system. The trick lies in finely balancing both and this is better said than done. Long term antibiotic prophylaxis for example with co-trimoxazole may be a useful adjunct therapy that could minimise risk of infection especially while on treatment with immunosuppressive drugs. Steroids are used as first line therapy in most autoimmune manifestations. Care should be used to use steroids in the lowest possible dose for the least period of time. Where the autoimmune

manifestations are severe, early use of second line agents must be considered. With better understanding of the underlying molecular defects, targeted therapies are being used; for instance use of abatacept(CTLA4-Ig) in CTLA4 and LRBA deficiency is an exciting development(1,14). Though not as perfect a solution, drugs causing cell cycle inhibition and predominantly affecting T cells (rapamycin, cyclosporine, mycophenolate), B cell blockade (rituximab)and TNF blockade are being used considering the underlying pathophysiology of each disorder. These drugs may be preferable over the non-specific action of glucocorticoids. Along with rectification of the primary immune defect, haematopoietic stem cell transplantation (HSCT) also improves autoimmune complications in most cases (1,3). It is also important to realise that every manifestation need not be treated with immunosuppression.

Systemic autoimmune disease such as RA,SLE, vasculitis, inflammatory myositis and scleroderma are seen in a minority.These need to be treated as per standard recommendations for these diseases.At the same time, caution needs to be exercised and the immune defect that predisposes to increased infections should be factored in while taking management decisions. Mono, oligo, or polyarticular arthritis has been described in PIDs. Juvenile idiopathic arthritis has been reported in XLA, CVID, sIgAD, HIgM, WAS and ALPS.As infectious arthritis is also common in these situations, it is important to make the distinction. For instance, arthritis in XLA mostly involves lower extremity large joints and is seen in 15% to 20% of patients. As opposed to destructive arthritis caused by infection like mycoplasma, most of the time arthritis is mild and responds to adequate immunoglobulin replacement therapy (15). This raises the possibility of an underlying atypical/subclinical infection stimulating synovitis. In case of suspicion of Mycoplasma, a course of macrolide antibiotic can be considered.

Hydroxychloroquine can be prescribed case of infectious or autoimmune. However, prior to starting other immunosuppressives, synovial biopsy,synovial fluid and tissue microbiological analysis and histopathology must be done. HLA typing may be of some value. HLADR1 and DR4 positivity is seen in JIA,HLA B27 in spondyloarthritis, HLADRB1 in RA, HLADR2 and HLADR3 in lupus.

Skin involvement is common in PIDs and many times provide a valuable clue in diagnosing type of PIDs.After infection, the most common skin condition in patients with PIDs is eczema seen in one-fifth of patients. Eczema is seen in Hyper IgE syndrome, Wiskott–Aldrich syndrome (WAS), and IgA deficiency(16). Alopecia and erythroderma is a characteristic feature of Omenn syndrome. Psoriasis, vitiligo, alopecia and lichen planus are other skin manifestations seen. Psoriasis is seen in CVID and IPEX whereas vitiligo has been reported in CVID,sIgA deficiency and APECED. Mainstay of treatment of these conditions is topical therapy including steroids and immunosuppressants.

As autoimmune cytopenias, lung disease and gut involvement are most commonly encountered, management and treatment of these shall be discussed briefly.Cytopenias in PIDs can be multifactorial. Apart from autoimmune causes, peripheral sequestration due to splenomegaly or bone marrow infiltration arising from infection or malignancy can cause cytopenia. Cytopenias can also be drug induced. Evaluation for multilineage cytopenia may require bone marrow biopsy. Steroids are often the first line agents used in the setting of autoimmune hemolytic anemia and autoimmune thrombocytopenia. Dose of steroids varies from 0.5 mg/kg-1 mg/kg.In some instances especially in severe disease, 2 mg/kg or pulse steroid may be required. IVIG in immunomodulatory dose may offer a valuable solution in a patient at higher risk for

infection. However, high cost may be a deterrent to the use of IVIG. Splenectomy is being less used now as it is invasive and predisposes to higher risk of infection.Other immunomodulatory agents such as azathioprine, mycophenolate , cyclosporine, sirolimus, rapamycin have been used. For thrombocytopenia, thrombopoietin receptor agonists such as eltrombopag is also an available option. Biologics such as anti-CD20 monoclonal antibody rituximab and eculizumab are also available though this need to be very clearly balanced with risk of infection.WAS and combined immunodeficiencies with autoimmune cytopenias are unlikely to respond to these agents and may be an added indication for HSCT in these patients (14). In ALPS, sirolimus is used for lymphadenopathy and splenomegaly. Sirolimus possibly acts by inducing apoptosis and eliminating double-negative T lymphocytes. In patients with ALPS and other PIDs with isolated chronic neutropenia(absolute neutrophil count<500) and associated infections, low-dose (1-2 μg/kg) G-CSF has been given twice or thrice weekly, subcutaneously(17).

Lung disease in PIDs can have varied etiologies- infection, malignancy and autoimmunity. Lymphocytic interstitial pneumonitis is seen in CTLA4 deficiency. One characteristic finding that is seen is CVID and LRBA deficiency is granulomatous and lymphocytic interstitial lung disease (GLILD). Non-necrotising granuloma, lymphocytic interstitial pneumonitis, and follicular bronchiolitis are the key histopathological features. GLILD is generally considered as the pulmonary manifestation of a systemic inflammatory phenotype associated with autoimmune cytopenia, splenomegaly, enteritis, and adenopathy(18). Patients with ILD usually present with non-specific symptoms such as coughing and breathlessness.Chest radiography and high-resolution computed tomography (HRCT) helps to delineate the type of interstitial lung disease.Findings of lung

nodule, ground glass opacities, and adenopathy are suggestive but not confirmatory.In all cases of ILD, possibility of infection needs to be ruled out. For example mycobacterial and fungal infections causing granuloma could mimic GLILD. Bronchoscopic alveolar lavage, microbiological investigation and lung biopsy may be required in select cases in case of diagnostic confusion or in case of inadequate response to therapy. PFT is useful for functional assessment of the degree of restriction and also for monitoring response to therapy.Apart from corticosteroids and immunomodulators, combination of rituximab and azathioprine has been used successfully in GLILD (19) . Lung biopsy can also guide management decision that is use of B cell blockade (rituximab) and T cell inhibition (rapamycin,cyclosporine ,mycophenolate) based on the predominance of the cells in biopsy. Apart from immunomodulation, optimisation of IVIG therapy and use of antimicrobials is strongly recommended (1).

Gastrointestinal manifestations are common in PIDs as gastrointestinal tract is the largest lymphoid organ. Autoimmune/inflammatory GI disorders in PID consist of autoimmune enteropathy, inflammatory bowel disease, pernicious anemia, celiac disease and granulomas. Needless to say, it is often difficult to differentiate these conditions from infectious GI disorders. Autoimmune/inflammatory GI manifestations are commonly seen in CVID, CGD, IPEX and IPEX-like disorders, XIAP deficiency, IL-10 and IL-10 receptor deficiency, Omenn syndrome, NEMO deficiency, WAS and XLA. Autoimmune enteropathy is seen in 100% of patients with IPEX. In IPEX and CVID, patients present with profuse watery, non-bloody diarrhea. Whereas in CGD, XIAP deficiency and NEMO deficiency, a more inflammatory phenotype characterised by a friable bowel mucosa and bloody diarrhea are seen (1). Fistulating IBD with perianal disease is seen in CGD, XIAP deficiency and IL-10 deficiency

(20). IBD is seen in 40% of patients with CGD. Granuloma formation in CGD can cause obstructive symptoms.

A thorough history and clinical examination with attention to oral ulcers, anal fissures, peri-anal fistulas, abdominal tenderness and ascites is needed. Infectious causes including Giardia,Salmonella, Cryptosporidium, Clostridium difficile,cytomegalovirus and atypical Mycobacteria needs to be ruled out by stool studies, culture and PCR where available(20).Fecal calprotectin may be of value in differentiating inflammatory from non-inflammatory causes; though it is not confirmatory for inflammatory bowel disease. Nutritional status can be assessed by albumin and prealbumin, those with chronic diarrhoea and malnutrition should be assessed for nutrient deficiency (iron, folic acid, zinc, selenium,copper, magnesium, calcium, vitamin A, B12, E, and K). Cross-sectional imaging aids in localising the disease process. Endoscopies and biopsies provide vital clues. Histopathologic findings include granulomas, nodular lymphoid hyperplasia, villous atrophy of the small bowel, lymphocytic infiltrates, and eosinophilic infiltrates(1). Nodular lymphoid hyperplasia (NLH) is mostly seen in patients with CVID and antibody-deficiency syndromes other than agammaglobulinemia. Villous atrophy is the prominent histopathological finding in autoimmune enteropathy as in IPEX and CVID. Autoimmune enteropathy in CVID is associated with absent plasma cells. Eosinophilic infiltrates are seen in IPEX, CGD and CVID.

Prophylactic antibiotics and optimisation of IVIg must be considered and may be useful in many situations. Steroids are used as first line treatment in autoimmune enteropathy .While this may be sufficient in CVID and CGD, IPEX and Omenn syndrome require more aggressive treatment including upfront cyclosporine. HSCT improves bowel symptoms in most PIDs, but worsening of

IBD after HSCT is seen in NEMO deficiency. TNF- α antagonists improve IBD outcome in these patients (20). TNF blockade also shows improvement in XIAP deficiency but was associated with increased infections in CGD. CGD colitis is more of an autoinflammatory condition and hence improvement with IL-1 blockade is not surprising (20). Surgical intervention should be avoided in granulomas in CGD.

Celiac and celiac-like diseases are found in CVID and selective IgA deficiency. Unlike in immunocompetent patients, use of IgA anti-tissue transglutaminase can lead to underdiagnosis of celiac disease in patients with IgA deficiency. In these situations, IgG anti-tissue transglutaminase is advised. As villous atrophy can also be seen in patients with autoimmune enteropathy, genetic haplotype testing for HLA-DQ2 and HLA-DQ8 may be required to ascertain diagnosis of celiac disease (21). Further, it is to be noted that while sIgA deficiency patients with celiac disease respond to gluten free diet, patients with CVID are more likely to have celiac-like phenotype that necessitates added immunosuppressive treatment (21).

Conclusion

Autoimmune/inflammatory complications can be seen in one quarter of patients with PIDs.Prevalence varies widely and depends on the type of underlying PID. Disorders of immune dysregulation have autoimmunity in 100%. Multiple mechanisms can underlie PIDs. As autoimmunity/inflammatory features can be presenting features of immunodeficiency, it is suggested that these symptoms are included in the Jeffrey Modell warning signs of PIDs. Apart from autoimmune cytopenia, autoimmune/inflammatory lung and gastrointestinal manifestations are relatively more common. Systemic autoimmune disease such as SLE, RA, and JIA are also seen in a minority of

patients. Diagnosing autoimmune/inflammatory phenomenon in PIDs can be complicated. Serologic tests can be misleading in patients with hypogammaglobulinemia. Alternate diagnosis including infections must always be considered and ruled out. Treatment includes judicious use of immunosuppressants and close monitoring for infections and adverse effects of the immunosuppressive drugs. Knowledge of the underlying pathology and pathogenesis guides selective and rational use of immunosuppressive agents.

Abbreviations used

AID: Activation-induced cytidine deaminase
AIHA: Autoimmune hemolytic anemia
AIRE: Autoimmune regulator
ALPS: Autoimmune lymphoproliferative disease
ANA: Anti-nuclear autoantibody
APC: Antigen-presenting cell
APECED: Autoimmunity–polyendocrinopathy–candidiasis–ectodermal dysplasia
BAFF: B-cell activating factor
CGD: Chronic granulomatous disease
CID:Combined immunodeficiency
CRP: C-reactive protein
CTLA4: Cytotoxic T-lymphocyte antigen 4
CVID: Common variable immunodeficiency
DOCK8: Dedicator of cytokinesis 8
FoxP3: Forkhead box P3
GOF: Gain-of-function
HSCT: Hematopoietic stem cell transplantation
IBD: Inflammatory bowel disease
IPEX: Immune dysregulation polyendocrinopathy enteropathy, X-linked syndrome
ITP: Immune thrombocytopenic purpura
IVIG: Intravenous immunoglobulin
JIA: Juvenile idiopathic arthritis
LIP: Lymphocytic interstitial pneumonitis
LRBA: LPS-responsive vesicle trafficking, beach and anchor containing protein

NRH:Nodular regenerative hyperplasia
PID: Primary immunodeficiency disease
PLC g2: Phospholipase C g2
RA: Rheumatoid arthritis
RAG: Recombination-activating gene
RF: Rheumatoid factor
SCID: Severe combined immunodeficiency
SLE: Systemic lupus erythematosus
STAT: Signal transducer and activator of transcription
Treg: Regulatory T

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General management of children with Primary immuno-deficiencies

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Primary immunodeficiency diseases (PIDs) are a growing group of over 230 heterogenous inherited disorders. People with PIDs are more prone to infections. In addition a poorly regulated immune system may attack tissues, leading to inflammation, and autoimmunity. Once recognized, these disorders are treatable and in some cases curable by BMT or gene therapy. Untreated PIDs are often chronic, serious, or even fatal. Infection has got a major role in determining the outcome of these patients. General measures to prevent infections, antimicrobial prophylactic therapy, immunizations and immunoglobulin replacement therapy (IRT) are some of the treatment modalities.

General measures against infections:
Hand and dental hygiene, good nutrition, avoidance of exposure to people who are ill with an infection, and withdrawal from school during periods of chickenpox and measles outbreaks are some of the precautions for immuno-deficient children.

Prophylactic antimicrobials:

Clearly defined prophylaxis is very important to prevent infections. Currently no standardized approach for prophylactic antibiotics is available due to the lack of randomized, controlled studies. The choice of antimicrobials is based on type of PID and the expected microbial susceptibility.

Table 1: Antimicrobials of choice and dosage schedule in various Primary immunodeficiency disorders in children

Disease	**Drug**	**Regimen**
Common variable immunodeficiency	Septran Azithromycin	5mg/kg Septran in 1-2 divided doses daily or 3 days/week 10mk/kg once daily
X-linked agammaglobulinemia	Septran	5mg/kg Septran in 1-2 divided doses daily or 3 days/week
Major Di George Syndrome	Septran Fluconazole	Same as above 3mg/kg once daily
Partial Di George Syndrome (CD4> 400)	No prophylaxis	
Leukocyte adhesion deficiency (LAD) type I	Amoxycillin/ Clavulanic acid or Fluoroquinolones	45 mg/kg/day 3 divided doses Individualised regime
Wiskott Aldrich Syndrome	Septran Acyclovir Fluconazole	Same as above 80 mg 4 times daily/kg 3mg/kg once daily

Chronic granulomatous disease	Septran Ciprofloxacin Itraconazole Interferon g	6mg/kg 1-2 times daily 5mg/kg once daily 5mg/kg once daily 50mg/m2 three times/week
Hyper IgE syndrome STAT3 deficiency If bronchiectasis: If pneumatoceles:	Septran Flucloxacillin Azithromycin Tobramycin Itraconazole	6mg/kg 1-2 times daily 125-250mg twice daily 10mg/kg once daily 300mg twice daily inhalation 5mg/kg once daily
Ataxia telangiectasia	Azithromycin	10mg/kg orally 3 days/week
INF-γ/IL-12 pathway defects	Azithromycin or Clarithromycin	10 mg/kg once daily

Table 2: Prophylaxis in SCID against specific pathogens:

Infectious Agent	**Drug**	**Regimen**
Respiratory syncytial virus	Palivizumab 15 mg/kg	IM Once monthly
Pneumocystis jiroveci	Septran (5 mg/kg of Trimethoprim)	1-2 divided doses daily,
Pneumocystis jiroveci	*Pentamidine isetionate 300 mg Inh	Once every 3 weeks
Pneumocystis jiroveci	*Dapsone 2 mg/kg	Once daily

Candida	Fluconazole 3 mg/kg	Once daily
Herpes family viruses	Acyclovir 80 mg/kg	Four times daily

If septran is not tolerated or not effective, give Pentamidine isetionate nebulization against PCP, Dapsone is another alternative.

Special situations

1. *SCID and other severe T cell disorders:* Start broad-spectrum prophylaxis with antibacterial, antiviral and antifungal agents immediately and continue until hematopoietic stem cell transplantation (HSCT). While giving blood products use irradiated (5000 RADS), CMV-negative, leukocyte depleted, especially if planning for BMT. Strict isolation to be followed. The child should ideally be managed in a laminar air flow facility, by staff immune to varicella and influenza and should receive sterile feeds. Palivizumab during the RSV season in children under 2 years with a CD4+ count <200, on a monthly basis is advised. Withhold breast feeding until the CMV is ruled out in mother and child. If BCG is given at birth in SCID, start chemoprophylaxis with INH 10 mg/kg and rifampicin 10mg/kg.

2. *Hyper IgM syndrome:* Reduce the risk of cryptosporidium infection by avoiding contact with pets,drinking only boiled or filtered water, avoidance of swimming in ponds and lakes, use of swimming pool only when aged >5 years.

3. *X- Linked Agammaglobulinemi*a: There is unique susceptibility to enteroviral meningoencephalitis. So strict hand hygiene of caregivers and healthcare professionals, especially after diaper changing is advised to prevent enteroviral and enterococcal infections.

4. *Complement disorders:* Give letters, describing predisposition to systemic bacterial infection or autoimmune disease manifestations and their management approach to parent or patient, to be used by school, camp, or emergency room physicians.

5. *Wiskott Aldrich Syndrome:* Splenectomy is sometimes indicated in severe bleeding (when platelet count cannot be improved with other measures).

6. *Job syndrome:* Regular bleach or chlorhexidine soaks and intermittent mupirocin applications reduce the colonization of skin by staphylococcus aureus.

7. *Immunodeficiency and chickenpox:* Following exposure give, VariZIG, within 48 hours of exposure. Ig replacement therapy is an alternative option. If the child already has a vesicular rash, treat with intravenous acyclovir.

8. *Contact with influenza-like illness:* If the child is not effectively protected by influenza vaccine, start oseltamivir prophylaxis within 48 hours.

9. *Prophylactic antibiotic treatment of bronchiectasis in children with PIDs:* Achieving a high trough IgG level along with antibiotic prophylaxis is useful to prevent infections and also progression of bronchiectasis. Macrolide antibiotics have anti-inflammatory as well as anti-microbial properties. Low dose azithromycin is effective in reducing the frequency and severity of airway pathogens, including Pseudomonas aeruginosa and mycoplasma. Azithromycin administration for 3 days, either consecutively or on alternate days each week, is efficient as a long-term prophylaxis. Inhaled tobramycin as aerosolized tobramycin is found to be effective in patients colonized with Pseudomonas.

Vaccination in children with PIDs:

Modify routine immunization of patient and their household contacts. Generally, avoid live virus vaccines like MMR, OPV, Varicella, MR, Measles, & Rotavirus except in complement deficiencies. Never give live bacterial vaccines like BCG and Ty21a in suspected severe T- cell disorders like SCID. Killed/subunit vaccines like HAV, HBV, conjugate vaccines (Hib, Typhoid & Pneumococcal) can be given. But their effectiveness is doubtful in predominantly B cell disorders. Trivalent non-live seasonal influenza vaccines should be offered during each influenza season. Vaccine administration should be deferred until at least 3 months after cessation of Ig replacement therapy, with the exception of influenza vaccine, which is indicated even if the patient is on IRT. Recommended and contraindicated vaccines in various PIDs is given in Table 3

Table 3: Recommended and contraindicated vaccines in various PIDs

B-lymphocyte defects	**Vaccine Contraindications**	**Vaccine Recommendations**
X-linked agammaglobulinemia (XLA)	All live vaccines	Pneumococcal conjugated, Haemophilus,meningococcal Trivalent influenza (non live)
Common Variable Immunodeficiency(CVID)	All live vaccines	Pneumococcal conjugated, Haemophilus,meningococcal Trivalent influenza (non live)
Selective IgA Deficiency	OPV	Pneumococcal conjugated, Haemophilus,meningococcal

		Trivalent influenza (non live)
IgG subclass Deficiency	None	Pneumococcal conjugated, Haemophilus,meningococcal Trivalent influenza (non live)
T-lymphocytes defects	Vaccine Contraindications	Vaccine Recommendations
Severe Combined Immunodeficiency	All live vaccines. Never give BCG	---
DiGeorge Syndrome(DGS)	All live vaccines except partial DGS	MMR, Varicella if CD4+>400
WAS	All live vaccines	Pneumococcal conjugated, Haemophilus, meningococcal Trivalent influenza (non live)
HIGM-CD40 Ligand deficiency, (Hyper IgM)	All live vaccines	Pneumococcal conjugated, Haemophilus, meningococcal Trivalent influenza (non live)
Phagocytic defects	Vaccine Contraindications	Vaccine Recommendations
Chronic granulomatous disease	Live bacterial vaccines(BCG, and Ty21a Salmonella typhi	Pneumococcal conjugated Haemophilus, meningococcal Annual non live influenza

**BCG is contraindicated in CVID but may be given in XLA*

Vaccination of household contacts:
OPV which is transmitted from person to person should be avoided, but MMR should be given to siblings, because transmission of vaccine viruses is not reported. Varicella vaccine can be given, because transmission of vaccine virus is rare, but it should be avoided in contacts of suspected SCID. Yearly influenza vaccination of family is recommended. In complement disorders immunize the family against all capsulated organisms.

Immunoglobulin replacement therapies (IRT):
Immunoglobulin replacement therapy is the cornerstone of treatment for antibody deficiency diseases, which is the most common group of PIDs. Immunoglobulin is administered by both intravenous and subcutaneous routes, but subcutaneous immunoglobulin preparations are presently not being marketed in India. Most patients will require this treatment indefinitely. Conditions indicated for Ig therapy are common variable immunodeficiency(CVID), X-linked hypogammaglobulinaemia (XLA), IgG subclass deficiencies and specific antibody defects. Consequently, patients with primary immunodeficiency other than exclusively antibody deficiency, like severe combined immunodeficiency(SCID), Wiskott-Aldrich syndrome(WAS) and ataxia telangiectasia may also benefit from IRT.

The goal of Ig replacement therapy is to prevent bacterial infections and avoid organ damage that leads to chronic disease and poor quality of life. Infection prevention, rather than a targeted serum IgG level is the goal of Ig replacement therapy as the protective serum IgG level varies with individual patients.

Commercially available preparations of these globulins are comprised of numerous IgG antibodies purified from blood or plasma donations from approximately 60,000 donors per batch. The name IVIG refers to the intravenous form of IG and SCIG, which is given subcutaneously. PID patients should be given priority in receiving Ig during scarcity and financial constraints. Immunoglobulin therapies are listed as Essential Medicines by the WHO.

The recommended starting dose of Ig replacement therapy is 400–600 mg/kg/4 weeks for the IV formulation and 100–150 mg/kg/week for the subcutaneous formulation. Trough levels should be assessed regularly and dose to be adjusted depending upon the frequency of infection. Suri et al, demonstrated that a median trough IgG level of 354 mg/dl was protective for majority of the children. Lower trough levels have been associated with the progression of chronic lung disease in otherwise asymptomatic patients. Dose should be increased if there are signs of changing lung function or if the patient continues to experience recurrent infections.

During an infection, the antibodies to that infectious agent are rapidly used up, so there may be a need for additional amounts of Ig during that illness. Ig may also provide broad protection against infections that may occur during invasive surgery. So extra Ig during infections, such as pneumonia, and during surgery can be given. Appropriate antibiotic coverage should also be considered during surgery.

The most common adverse events associated with this therapy are headache, flushing, chills, myalgia, wheezing, tachycardia, lower back pain, nausea, and hypotension

Interferon-gamma therapy:
Used to treat chronic granulomatous disease.

When to start antibiotic prophylaxis and IRT:
In mild cases, start prophylaxis when infection rate exceeds three per year or following a very severe infection. IgA deficiency or transient hypogammaglobulinemia of infancy needs a short course of prophylaxis only. For specific antibody deficiency, first choice is prophylaxis and if there is no response, start immunoglobulin replacement therapy (IRT). In the case of severe antibody deficiency, IRT is the first choice, prophylaxis added if IRT is not effective.

Hearing test & pulmonary function test:
Since B-cell immunodeficiency disorders are often associated with hearing loss and pulmonary complications, regular hearing assessments and monitoring of pulmonary function is recommended. As with primary T-cell defects, vigilance for malignancies and autoimmune disorders is also important in patients with B-cell disorders.

Newborn screening (NBS) in PID:
HSCT is a curative treatment for several PIDs and the best outcome is achieved if transplant is performed before these children acquire an infection. SCID, which is not apparent at birth can only be detected by NBS. NBS for SCID is done using T cell receptor excision circles assay (TREC assay), which is a measure of thymic output of T cells and is very sensitive. NBS for SCID is now being routinely carried out in USA and many other developed countries. In addition to SCID the assay would also identify infants with T cell lymphopenia due to other primary and secondary causes. So a low TREC levels have been detected in individuals with 22q deletion syndrome, CHARGE association,

and Trisomy 21. In addition, infants with forms of PID other than SCID may have low TREC, for example in ataxia telangiectasia and combined immunodeficiency diseases (CID).

Screening for B Cell Deficiency:
B cell antigen receptors, extranuclear circular DNA called kappa recombining excision circles (KREC) is used as B cell marker. This KREC assay using a PCR-based method can detect SCID, X-linked agammaglobulinemia (XLA) and CVID. A multiplexed TREC/KREC assay also possible.

Emergency medicine:
Primary immunodeficiency patients may need emergency treatment. So every patient should have an individualized plan regarding diagnosis during infections and autoimmune complications, specific therapy, expert center contact. A 24-hour contact number for specialist advice must also be included in the patient's plan. This is particularly important for patients with complement deficiencies in whom the correct emergency therapy saves lives.

Genomics of Genetic Diseases - Emphasis on Primary Immunodeficiency Disorders

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The availability of the complete reference human genome in 2003 has significantly enhanced our understanding of genetic diseases and the molecular defects causing them[1]. The decade which followed saw an unprecedented advancement in technologies which has enabled routine application of genomic technologies in clinical settings. With the significant reduction in costs of sequencing, genomic technologies are rapidly being deployed for molecular diagnosis, carrier screening as well as preimplantation and prenatal genetic screening[2].

Why a molecular diagnosis is important

Molecular diagnosis in a case of PID is important for the following reasons. Firstly it helps in the identification/confirmation of the molecular defect and therefore enables an appropriate and evidence based course of action. Secondly, since many of the genetic conditions occur in higher frequency in the background of endogamy or consanguinity, it enables evidence based genetic counselling and carrier screening of prospective parents. Prevention of genetic disease through appropriate evidence based genetic counselling and carrier screening is one of the most cost-effective interventions to prevent the new incidence of most

genetic diseases. Thirdly, identification of the genetic defect enables pre-implantation / prenatal genetic screening[3].

Genetic Counselling

It should however be emphasised here that genetic/genomic testing should always be accompanied by a pre and post test genetic counselling to explain the test in detail and also prepare the individuals being tested (typically prospective couples) on the outcomes of the test and the chances of positivity. In addition, the chances of finding incidental genetic variants and potential impact should also be discussed if the genetic testing / screening test reports such incidental findings as a policy. It should be noted that even in the best case scenario, even genome sequencing would not be able to provide a 100% positivity, due to the fact that a significant number of genetic variations causing diseases are yet to be understood[4].

Extreme caution needs to be taken in revealing the inheritance pattern , especially in case of X-linked disorders to the extended family and community in societies where such revelation is likely to result in discrimination.

It needs to be emphasised that genetic counselling is a discussion between the patient and the treating clinician. The clinician is expected to lay out all the options, their advantages and disadvantages and the possible outcomes and risks and associated costs of the primary and secondary investigations and their long-term implications. Rather than suggesting an approach, the approach should be to urge the patient to come up with a socially, culturally and economically appropriate way forward[5].

With regard to selection of samples for genomic testing, it is technically advisable to always perform the test on the trio (index

case + parents) which provides significantly higher positivity. Nevertheless, in situations where cost considerations are of significance, testing for the index case only is the approach widely practiced.

Options of genetic tests available

A number of genetic test options are available . This includes single gene tests, gene panels as well as whole exome and whole genome sequencing along with other specialized tests for small and large chromosomal deletions/duplications[6].

Single gene tests are typically employed for single gene disorders. The principle of a single gene test is to be able to sequence the protein coding exons of a gene. Single gene tests are employed for instance in X-Linked agammaglobulinemia, caused by mutations in BTK gene. A near confirmatory diagnosis of XLA on clinical workup would mandate the molecular testing. Similar is the case of X-linked Hyper IgM syndrome or Hyper IgD syndrome, where the genetic defects are mapped to the CD40L and MVK genes respectively. It should however not be taken for granted that all single gene disorders need to be tested using a single gene test, as the cost effectiveness may not be high especially if the gene is large in size as it require multiple primer set to perform sequencing which incur large amount of money. Therefore, the selection of the test should also depend on the availability, accessibility and affordability of the test and more importantly the accuracy in clinical diagnosis, without which a considerable amount of precious time is lost in pursuit of a molecular diagnosis.

Mullti gene / Gene panel tests are employed when the disease could be caused by mutations in any of the multiple genes. Typically, gene panel tests are employed when there are a handful of genes implicated in a disease, like CVID. Panel testing is

increasingly being replaced by exome sequencing due to the cost effectiveness and diagnostic positivity.

Exome sequencing is employed typically when there are a large number of genes involved in the disease. This option also needs to be considered if the patient doesn't have clinical features characteristic of a known PID or when multiple differentials need to be considered. Whole exome sequencing typically uses next generation sequencing to cover exonic / protein coding regions of over 20,000 genes. It has therefore emerged as a cost effective approach for molecular diagnosis of genetic diseases. Best examples of such diseases would be Severe combined immunodeficiency (SCID) where mutations in at least 37 genes could cause the disease[7].

Whole genome sequencing is currently an emerging diagnostic test. WGS is faster, and covers the entire genome, compared to the 2% of the genome covered by whole exome sequencing. In addition, WGS enables identification of a number of types of genetic variations including chromosomal abnormalities, duplications/deletions apart from single nucleotide variants. Due to the high cost, this testing is currently reserved only for undiagnosed genetic disorders. However as costs of whole genome sequencing is rapidly decreasing, WGS is expected to replace a whole lot of genetic tests, including panel as well as exome sequencing in coming years[8].

Other tests:

T-cell receptor excision circles (TREC) and kappa-deleting recombination circle (KREC) assays are used in some centres as a quick screening tool for patients suspected to have a B or T cell defect. The TREC/KREC assay offers a quantitative assay for the lymphocyte populations. While not routinely used in most

developing countries,10, the limited availability of flow cytometry and cost considerations make TREC/KREC a promising screening tool in settings which are resource limiting[11].

In a small number of cases, genomic deletions are the cause of disease. For example, X linked Agammaglobulinemia caused by deletions in BTK gene[12]. Such cases are typically difficult to be identified on exome or panel testing and therefore specialised tests are required. The most popular test for small genomic deletions is Multiplex Ligation-dependent Probe Amplification (MLPA) in short, which can detect small deletions/duplications[12,13]. Large deletions are typically identified using chromosomal microarrays (CMA) or array Comparative Genomic Hybridisation (array CGH)[14].

Interpreting a Genomic Test

According to the recent guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (AMP), all variants associated with a disease are classified in one of the five groups - Pathogenic, Likely Pathogenic, Variants of Unknown Significance (VUS), Likely Benign and Benign. This classification follows a systematic analysis of a number of parameters, population frequency of the genetic variants as well as published literature on the effect of the genetic variant as evidenced by experiments in the lab[15].

Report with a Pathogenic / Likely Pathogenic Variant

The test is considered positive when the report has at least a variant in the Pathogenic / Likely Pathogenic category and matches the zygosity as expected for the disease. If further action is planned on the report, for example a prenatal testing or pre-implantation genetic screening, it would always be wise to validate the variant using Sanger sequencing (if the original investigation was on Next

Generation Sequencing) so that precious time is not lost in standardizing the assay at a later point in time. The capillary sequencing assay would also be handy as a cost effective option for screening additional family members / community who are in need.

While the identification of the Pathogenic variant ends the diagnostic odyssey for the patient/family, more importantly publication of the variant in a public resources like ClinVar, VariantShare (see below) or in peer reviewed publications would ensure that a number of patients and families would benefit from a faster diagnosis.

Report with Variant of Unknown Significance

While a clinical decision can be made without much of an issue in the case of pathogenic or likely pathogenic variant, the dilemma arises when the variant is reported as a VUS. a VUS essentially means there is not enough evidence to classify the variant to either sides of the classification spectrum. In many cases, additional evidence can come from an extended pedigree or additional members in the family and if possible affected individuals who could be screened for the variant. Alternatively population control databases for Indian/Asian populations like SAGE http://clingen.igib.res.in/sage could be used to ascertain the population frequencies[16]. Resources like VariantShare http://clingen.igib.res.in/variantShare/ allows clinicians and patients to share variants along with the phenotypes to ascertain whether additional matches with the same variants and clinical phenotypes were observed elsewhere in the community. In some cases, despite the best of efforts the variant classification is VUS, which will require experimental evidence which is usually laborious and time consuming.

Negative Report

The positivity of the genomic test varies significantly depending on the disease, the clinical workup and phenotypes provided for genetic correlation as well as experience of the centre in addressing similar diseases and variants and underlying databases and resources used.

A negative genomic report does not mean the end of the tunnel in the diagnostic odyssey. It just means that one has lost one's way.

There are a number of options in hand before losing hope

Re-evaluate the raw data (FASTQ files) and variant files (VCF files) at a centre experienced in working up cases of PID. Subtle changes in the analytic approach and a keen eye for typical variants missed in regular analysis can sometimes reveal variants which can otherwise be missed.

Re-evaluate the data at a later point in time. As the understanding of the molecular mechanisms and the repertoire of genes and genetic variants causing diseases improves over years , re-analysis of the data at a later point in time has been shown to provide positivity as high as 15%. Nevertheless it is important to have access to the raw sequence files and the variant files handy[17,18].

If other types of variants like deletions/duplications are widely reported for the disease, re-evaluation using MLPA or CMA for the genes is advised if re-evaluation of the data provides clues to such variants.

Upgrade to a whole genome sequencing, as single gene/panel or whole exome sequencing can miss subtle variations in regulatory regions and certain types of variants like structural variants. Given

cost considerations, this should be the last resort of investigation at this point in time[19].

Screening for early diagnosis and prevention

Carrier screening is an option to be considered and actively advocated in communities where the social practice of endogamy or consanguinity is prevalent. Educational material and public discourses on disease and options to diagnose, treat and prevent the disease would be the mainstay to increasing awareness among the community members. It would always be wise to educate and involve the community leaders in such efforts[20]. All consenting adults should be counselled (see section above). There is no proven advantage of screening children unless the disease manifestation is marked by late onset, like Common Variable Immunodeficiency (CVID).

Identification of the molecular defect can enable Prenatal and Pre-implantation genetic screening. The choice of the approach are largely determined by the availability of local expertise, cost considerations and social, cultural and religious considerations. Pre-implantation genetic testing involves screening of the embryos after an in-vitro fertilisation procedure in an attempt to implant a disease free embryo. While this is culturally acceptable by most religions, the prohibitive cost and limiting expertise and resources makes it a much less sought approach. Prenatal testing involves amniocentesis or chorionic villus sampling and testing for the molecular defect. The skills and expertise are widely available and less costly and therefore the most widely sought approach on date.

Newborn screening for immunodeficiencies with T or/and B cell defects is in practice in some countries. This screening employs quantification of T-cell receptor excision circles (TREC) and kappa-deleting recombination circle (KREC). This may be

considered in families with a previous history of PID in the extended family or where frequency of SCID is high in the population. Early screening affords early diagnosis and appropriate treatment, without loss of precious time, resources and can avoid significant morbidity[9,21].

Summary and Conclusions

Genomics offers a variety of approaches for diagnosis and prevention of genetic diseases. With the decreasing costs, increasing awareness, accessibility and acceptability, genomic testing is increasingly being used in clinical settings for molecular diagnosis of PIDs. Continuous advancements in the field has enabled the identification of new genes and genetic variants associated with PIDs globally and in India and will significantly impact the early diagnosis while also offering an opportunity to prevent the disease.

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Stem Cell Transplantation in PID

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Allogeneic hematopoietic stem cell transplantation (HSCT) has been a curative option for several primary immune deficiencies (PID) including severe combined immune deficiency (SCID), Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD), hemophagocytic lymphohistiocytosis (HLH) and many other immunodeficiency disorders for over 47 years [1, 2]. The entire field of hematopoietic stem cell transplantation has progressed and evolved because of the ability to apply new transplant concepts in the treatment of patients with primary immunodeficiency disorders and the majority of innovations in the field of transplantation have been inspired by children with PID. These include the first allogeneic human leukocyte antigen (HLA)-matched sibling bone marrow transplants for severe combined immune deficiency (SCID) and Wiskott Aldrich syndrome (WAS) in 1968 [1, 2], adenosine deaminase deficiency and SCID (ADA-SCID) in 1975 [3], unrelated donor transplant in 1977 [4] and haploidentical related donor transplants in 1983 [5]. In 1981 busulfan and cyclophosphamide were used in the conditioning of patients with WAS. This then became the backbone of myeloablative transplant for other non-malignant disorders like hemoglobinopathies and inborn errors of metabolism [6]. These initial steps have opened the path to accepting HSCT as the treatment of choice in many forms of PID [7–11].

The process of HSCT involves stabilizing the child, identifying a suitable histocompatible donor, conditioning chemotherapy so as to enable the recipient to accept new stem cells, infusion of donor stem cells into the recipient's central vein, providing optimal supportive care for 2 to 3 wk until the new stem cells begin to grow and differentiate and maintain on immunosuppression for about 6 mo to prevent graft rejection and graft vs. host disease. PID transplants pose several specific challenges in each of these steps and these will be discussed in detail.

Severe Combined Immune Deficiency (SCID)

SCID is a syndrome of diverse genetic causes characterized by profound deficiencies of T- and B-cell function and, in some types, also of NK cells and function. On the basis of data obtained from eleven U.S. newborn screening programs in the general population, Kwan et al. reported an incidence of SCID of 1 in 58,000 live-births [12]. In a microarray sequencing of known 240 cases of SCID, a total of 153 distinct mutations were found and 87 (64 %) were found only once in this retrospective cohort.

Since the first successful bone marrow transplant performed in 1968, allogeneic HSCT is the standard treatment for all forms of SCID with over 80% long term survival when an HLA matched sibling donor is available and the majority of the children survived even in alternate donor transplants [13]. A retrospective study of 240 infants with SCID transplanted between 2000 and 2009 revealed that 5-year survival and T and B cell recovery were more likely in transplants from matched sibling donors than from alternate donors. However, the survival rate was high regardless of donor type among infants who received transplants at 3.5 mo of age or younger (94 %) and among older infants without prior infection (90 %) or with infection that had resolved (82 %). Among actively infected infants without a matched sibling donor,

survival was best among recipients of haploidentical T-cell-depleted transplants in the absence of any pre-transplant conditioning. The study concluded that transplants even from donors other than matched siblings were associated with excellent survival among infants with SCID identified before the onset of infection [14].

Wiskott Aldrich Syndrome (WAS)

WAS is an X-linked disease due to mutations in the WAS gene causing life-threatening primary immunodeficiency, thrombocytopenia, eczema and a high incidence of autoimmunity and malignancy. The WAS gene provides instructions for making a protein called Wiskott Aldrich Syndrome Protein (WASP) in all blood cells. Lack of any functional WASP results in decreased ability to form immune synapses and leads to immune dysfunction [15]. Currently, HSCT is the only potential curative therapy for WAS [16, 17], and the significant host immunologic barrier mandates the use of conditioning regimen prior to transplantation. CIBMTR/NMDP reported transplant outcomes of 170 boys with WAS where most patients were younger than 5 y (79 %), and received pre-transplantation preparative regimens without radiation (82 %) and had non-T-cell depleted grafts (77%). The 5-year probability of survival for all subjects was 70 %. The probabilities differed by donor type with 87 % in patients with HLA-identical sibling donors, 52 % in those with other related donors, and 71 % in those with unrelated donors [18]. A second study reported the results on 194 boys transplanted with 79 matched sibling donors, 91 unrelated donors and 24 unrelated cord blood with improved outcomes after unrelated donor transplant compared with historical experience. The survival of recipients over 5 years was inferior to that in the under 2 y of age at 73.3 vs. 91.1 %. Umbilical cord blood transplant recipients had poorer survival as their transplants were associated with a higher

risk of posttransplant complications including graft failure, GVHD (Graft vs. host disease), autoimmunity or malignancy. Lineage specific chimerism was unstable in the first-year post transplant in 20 % of patients, and mixed chimerism was more frequent among recipients of unrelated donor transplants. Myeloid chimerism of more than 50 % was generally associated with platelet counts above 50,000/ml [19]. In patients with WAS, mixed chimerism appeared to have a detrimental effect on event-free survival after HSCT due to an increased incidence of autoimmunity [20].

Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) was first described in 1954 [21, 22] as recurrent infections occurring in the setting of hypergammaglobulinemia. Intact NADPH oxidase is essential for intracellular killing of microorganisms. Multiple separate proteins contribute to intact NADPH oxidase, mutations in five of which lead to a single syndrome CGD [23]. Originally thought to be an X-linked disease, its recognition in girls in 1968 led to the determination of autosomal recessive forms as well [24]. Individuals who have autosomal recessive forms of CGD may also have other subtle abnormalities, such as vascular disease, diabetes and inflammatory bowel disease [25–27]. Over almost 60 years CGD has evolved from a disease of early fatality to one of effective management with high survival.

Bone marrow transplantation can lead to stable remission of CGD. Conditioning regimens ranging from full myeloablation to non myeloablative conditioning have lead to the cure of CGD [28, 29]. Even in the setting of refractory fungal infection, bone marrow transplantation has been effective with non-myeloablative transplants being more successful in children with other comorbid features [30, 31]. In patients transplanted with appropriate

conditioning regimen before the onset of serious infection, the outcome has been remarkable. With these encouraging results the transplant procedure is being extended for haploidentical grafts following α β T cell depletion with successful outcomes.

Issues in HSCT Specific for PID

The main controversy is related to conditioning – do SCID babies need to receive myeloablative therapy? Conditioning results in complete correction of T cells and B cells whereas non-conditioned child may only have T cell engraftment and continue to require lifelong IVIG replacement therapy. Is myeloablative therapy or reduced intensity treatment better for other PID? Myeloablative therapy increases the chance for complete chimerism but increases the risk of acute morbidity and mortality. Reduced intensity conditioning is less
toxic but may lead to mixed chimerism. For some diseases like CGD, mixed chimerism will be adequate to correct the disease. However, mixed chimerism may lead to development of autoimmune diseases in WAS. The optimum conditioning regimen must be evaluated on the basis of diagnosis, disease status, co-morbid features and type of donor.

New Developments in the Treatment of PID

The use of haploidentical donors has helped to offer HSCT even to children with no matched donors. Haploidentical transplantation has been specifically used to treat SCID since the 1980's [5]. However, newer techniques are now being used to improve the process of depletion of T cells and reconstruct immunity by being more selective in depletion ofαβT cells [32] and depletion of CD19+cells. In a study of T cell depleted haploidentical HSCT, the patients had rapid hematological recovery after transplant with a 22 % risk of GVHD and 27 % chance of graft failure. Optimal conditioning, the use of mega

doses of stem cells >10 Å~ 106/kg CD34+ cells and residual γ δ T cells and NK cell in the graft with no ongoing immunosuppression have resulted in excellent outcomes in this type of SCID transplant.

HSCT for PID in India

Primary immune deficiency disorders (PID) are an important cause of mortality in infants and children in India. There is little published data regarding the incidence and outcome of HSCT for PID from India. The main challenge faced so far is the lack of recognition of PID by primary care physicians and the lack of awareness on the success of HSCT for children with PID. This has resulted in very few referrals to tertiary care centres and hence only a handful of transplants for such a large country.

Most children are referred with disseminated BCG infection as BCG vaccination is mandatory on the day of birth. Poor general nutrition, the increasing incidence of hospital and community acquired drug resistant bacteria, lack of access to sophisticated molecular techniques to risk stratify these children and provide early therapy for viral infections have been common challenges faced in the care of these children.During immune reconstitution, BCG infection and cytomegalovirus (CMV) reactivation have been the main causes of morbidity and mortality. Ninety five percent of the population in India is CMV positive and CMV reactivation has been a major challenge in PID transplants, particularly in the alternate donor setting and, in those children, who had received granulocyte transfusions to overcome their infections in the peritransplant period.

SCID transplants need to be planned based on the molecular diagnosis. Due to lack of universal access to genetic diagnosis, a flow cytometry-based approach to conditioning has been adopted.

Babies with T negative, B positive, NK negative SCID can be transplanted early with no conditioning and good success rates. However, babies with T negative, B negative, NK positive SCID could possibly have DNA repair defects and have a better outcome with a treosulphan based reduced intensity conditioning. Late death due to extensive fibroelastosis in a SCID baby with 100 % donor and good immune reconstitution has been seen in one such child in the second authors' centre. Targeted busulphan is essential for transplanting young babies with immunodeficiency and is not available in all centres in India and may account for inferior outcomes with myeloablative regimens using busulphan without therapeutic drug monitoring. All centres had used a myeloablative conditioning protocol which included busulfan and cyclophosphamide until 2011, after which a fludarabine based reduced intensity conditioning (RIC) protocol with either busulphan or treosulphan was adopted.

Long term immunoglobulin replacement is expensive and not feasible in India. Hence, children with X-linked agammaglobulinemia with a matched sibling donor have been offered HSCT. The average cost of HSCT in India is about 15,00,000 rupees for sibling allograft and twice the amount for unrelated transplants. T cell depletion techniques are expensive. Hence, there is an increasing use of post transplant cyclophosphamide, based on the John's Hopkins protocol, following fludarabine and melphalan conditioning using a haplo matched family donor.

A total of 104 PID transplants have been done in the country as per data obtained from 10 participating centres that have performed PID transplants. Over half of these children are alive and doing well. This data reinforces the fact that PID can be treated successfully in developing countries and outcomes are

excellent with reduced intensity conditioning regimens. The outcomes in related transplants are excellent with low morbidity and mortality with good long-term outcome. Extended family typing should be done before embarking on an unrelated donor search for children with PID. Outcomes in unrelated transplant will improve with new cord blood and donor registries developing in India.

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