INDIAN JOURNAL OF PRACTICAL PEDIATRICS

- **IJPP is a quarterly subscription journal of the Indian Academy of Pediatrics committed to presenting practical pediatric issues and management updates in a simple and clear manner**
- **Indexed in Excerpta Medica, CABI Publishing, Scopus**

Vol.23 No.2 **APR.- JUN. 2021**

Dr.S.Thangavelu
Editor-in-Chief

Dr.T.L.Ratnakumari
Executive Editor

CONTENTS

TOPIC OF INTEREST - "IMMUNOLOGY"

Journal Office and address for communications: Dr. S.Thangavelu, Editor-in-Chief, Indian Journal of Practical Pediatrics, 1A, Block II, Krsna Apartments, 50, Thamizh Salai (Halls Road), Egmore, Chennai - 600 008. Tamil Nadu, India. Tel.No. : 044-28190032 E.mail : ijpp_iap@rediffmail.com

FOR YOUR KIND ATTENTION

* The views expressed by the authors do not necessarily reflect those of the sponsor or publisher. Although every care has been taken to ensure technical accuracy, no responsibility is accepted for errors or omissions.
* The claims of the manufacturers and efficacy of the products advertised in the journal are the responsibility of the advertiser. The journal does not own any responsibility for the guarantee of the products advertised.
* Part or whole of the material published in this issue may be reproduced with the note "Acknowledgement" to "Indian Journal of Practical Pediatrics" without prior permission.
* The write up should be in accordance with the recommendations of Central IAP particularly with issues involving National Programmes like Immunization, Public Health Programs and Nutrition.
* NOTE: Many trade names of the vaccines are included in the text for the sake of clarity.

EDITORIAL BOARD

Published by Dr. S.Thangavelu, Editor-in-Chief, IJPP, on behalf of Indian Academy of Pediatrics, from 1A, Block II, Krsna Apartments, 50, Thamizh Salai (Halls Road), Egmore, Chennai - 600 008. Tamil Nadu, India and Printed by Mr. D.Ramanathan, at Alamu Printing Works, 9, Iyyah Street, Royapettah, Chennai - 600 014.

INSTRUCTIONS TO AUTHORS

General

Print the manuscript on one side of standard size A4, white bond paper, with margins of at least 2.5 cm (1") in double space typescript on each side. Use American English using Times New Roman font 12 size. Submit four complete sets of the manuscript.

They are considered for publication on the understanding that they are contributed to this journal solely.

All pages are numbered at the top of the right corner, beginning with the title page.

All manuscripts should be sent to: The Editor-in-Chief, Indian Journal of Practical Pediatrics

Manuscript

1st Page –

Title

Name of the author and affiliation

Institution

Address for correspondence (Email, Phone, Fax if any)

Word count

No. of figures (colour / black and white)

No. of references

Authors contribution

2nd Page –

Abstract (unstructured, not exceeding 100 words) with key words (not exceeding 4)

3rd Page -

Acknowledgement

Points to remember (not more than 5 points)

Text

References

Tables

Legends

Figures – should be good quality, 4 copies black & white / colour,*

(4 x 6 inches – Maxi size) Glossy print

* Each colour image will be charged Rs. 1,000./- separately, with effect from January 2006 (Except for invited articles).

Text

Only generic names should be used

Measurements must be in metric units with System International (SI) Equivalents given in parentheses.

References

Recent and relevant references only

Strictly adhere to Vancouver style

Should be identified in the text by Arabic numerals as superscript.

Type double-space on separate sheets and number consecutively as they appear in the text.

Articles without references / defective references will entail rejection of article.

Tables

Numbered with Roman numerals and typed on separate sheets.

Title should be centered above the table and explanatory notes below the table.

Figures and legends

Unmounted and with figure number, first author's name and top location indicated on the back of each figure.

Legends typed double-space on separate sheet. No title on figure.

All manuscripts, which are rejected will not be returned to author. Those submitting articles should therefore ensure that they retain at least one copy and the illustration, if any.

Article Categories

Review article

Article should be informative covering the recent and practical aspects in that field. Main articles can be in 2500 - 3000 words with 15 - 20 recent references and abstract not exceeding 100 words.

Case report (covering practical importance)

250 - 600 words, 8 - 10 recent references

Clinical spotters section

150 - 200 words write up

With 1 or 2 images of clinically recognizable condition

(of which one could be in the form of clinical photograph / specimen photograph / investigation)

Letters to the Editor

200 - 250 words pertaining to the articles published in the journal or practical viewpoints with scientific backing and appropriate references in Vancouver style.

Check List

Covering letter by corresponding author

Declaration (as enclosed) signed by all authors **

Manuscript (4 copies)

Accompanied by a copy in CD / or submit as an email attachment in addition to hard copy.

Failing to comply with the requirement at the time of submission would lead to the rejection of the article.

Author's contribution / Authorship Criteria

All persons designated as authors should qualify for the authorship. Authorship credit should be based on substantial contributions to i) concept and design, or collection of data, or analysis and interpretation of data; ii) drafting the article or revising it critically for important intellectual content; and iii) final approval of the version to be published. All conditions 1, 2 and 3 must be met. Participation solely in the collection of data does not justify authorship and can be mentioned in the acknowledgement if wanted.

**** Declaration by authors**

I/We certify that the manuscript titled '…….' represents valid work and that neither this manuscript nor one with substantially similar content under my/our authorship has been published or is being considered for publication elsewhere. The author(s) undersigned hereby transfer(s), assign(s), or otherwise convey(s) all copyright ownership, including any and all rights incidental thereto, exclusively to the Indian Journal of Practical Pediatrics, in the event that such work is published in Indian Journal of Practical Pediatrics. I / we assume full responsibility for any infringement of copyright or plagiarism.

Authors' name(s) in order of appearance in the manuscript Signatures (date)

Selection procedures

All articles including invited articles will be peer reviewed by two masked reviewers. The decision of the Editorial Board based on the reviewer's comments is final. All manuscripts, which are rejected will not be returned to author. Those submitting articles should therefore ensure that they retain at least one copy and the illustration, if any.

IMMUNOLOGY

APPLIED IMMUNOLOGY AND CLINICAL CLUES FOR INBORN ERRORS OF IMMUNITY

*Geeta M. Govindaraj
**Kalpana George

Abstract: *Primary immune deficiency disorders or inborn errors of immunity are one of the commonest genetic disorders caused by variants in more than 400 genes. This is manifested by susceptibility to severe, persistent, unusual or recurrent infections encompassing a broad or narrow range of pathogens. Autoimmune, allergic and autoinflammatory manifestations and susceptibility to early onset malignancies may be the other manifestations. These disorders are often undiagnosed or misdiagnosed and enhancing awareness among pediatricians is key to improving outcomes, since a high index of suspicion is crucial.*

Keywords: *Primary immune deficiency, Inborn errors of immunity, Autoinflammation.*

Primary immune deficiency disorders (PIDDs) or inborn errors of immunity (IEIs) are a group of more than 400 rare inherited disorders that occur as a result of developmental or functional derangements in the immune system.[1] With the reduced burden of infectious diseases in children due to immunization and improved standards of living, the emphasis is shifting towards children who are at risk of death or disability from enhanced susceptibility to infections. Although rare individually, these disorders are much more common than they were once thought to be.[2] New achievements in this field have been possible due to collaborative efforts, improved immunologic techniques and use of next generation sequencing technology. Improved availability of life saving prophylactic therapies like intravenous immunoglobulin and treatment options like stem cell transplantation and gene therapy have given added impetus in making an early diagnosis.

* Professor,
Department of Pediatrics

** Associate Professor,
Department of Microbiology,
Government Medical College, Kozhikode, Kerala,
FPID Regional Diagnostic Centre, Kozhikode

email: geetakkumar@gmail.com

Applied immunology

Adaptive and innate immune defects

Primary immune deficiencies are a heterogenous group of inherited disorders that can affect either the innate or adaptive arms of the immune response and are usually the result of single gene mutations. They are often grouped based on the specific components of the immune system predominantly affected, namely, diseases affecting T cells (cell mediated immunodeficiency), B cells (humoral immunodeficiency), both B and T cells (combined immunodeficiency), phagocytic cells and/or NK cells (innate immunodeficiency) and complement components. This roughly correlate with the clinical presentation and the type of infection as well. However, due to the complex interactions of innate and adaptive immune systems, defects in one compartment can manifest in other arms of immune response.[3] Defects in different genes may have similar phenotype, whereas different variants in a single gene may result in unique phenotypes.[4]

The International Union of Immunological Societies (IUIS 2019) classification[1] lists ten groups of disorders

Box 1. Classification of inborn errors of immunity

1. Combined immunodeficiencies affecting cellular and humoral immunity
2. Combined immunodeficiencies with syndromic features
3. Predominantly antibody deficiencies
4. Diseases of immune dysregulation
5. Congenital defects of phagocyte number or function
6. Defects in intrinsic and innate immunity
7. Autoinflammatory diseases
8. Complement deficiencies
9. Bone marrow failure
10. Phenocopies of inborn errors of immunity

(Box 1) and includes the autoinflammatory syndromes as well as the inherited bone marrow failure syndromes.

Severe combined immune deficiency

Lymphoid progenitor cells originating from bone marrow reach the thymus and mature into functional T cells through sequential well defined stages. Severe combined immune deficiency (SCID) is characterized by mutations in various genes that have a role in T and B cell development and function and often involves the NK cells as well. Defective T cell function precludes the development of normal humoral immunity since antibody production by B cells is dependent on T cell help. The NK cells, a distinct subset of lymphocytes, may be normal in half the children with SCID and confer a degree of protection against bacterial and viral infections. In SCID with normal NK cells, the affected genes are those that encode for proteins involved in the development of a diverse repertoire of receptors on T and B cells by a process called V(D)J variable(V), joining(J) and in some cases, diversity(D) gene segments recombination.[5] This diverse repertoire of T and B cell receptors is necessary for recognition of a wide array of pathogens. Children with SCID usually do not survive beyond infancy and often have severe lymphopenia. Thymic hypoplasia, failure to thrive and recurrent / persistent thrush are characteristic.[6]

X - linked agammaglobulinemia

X - linked agammaglobulinemia (XLA) which is a prototypic humoral immunodeficiency is due to a pathogenic variant in the BTK gene. In this condition there is a block in B cell development at the pre - B cell stage in the bone marrow resulting in near total absence of B cells in peripheral circulation and markedly reduced levels of all immunoglobulin classes.[7] In common variable immunodeficiency, terminal differentiation of mature B cells is affected.

Wiskott - Aldrich syndrome

Wiskott - Aldrich syndrome is a rare X - linked recessive condition associated with syndromic features. The WAS protein (WASp) which plays an important role in cytoskeleton remodelling is defective and impacts on the formation of the immunological synapse between the T cells and the antigen presenting cells.[8] Defective T cell function leads to impaired B cell homeostasis with resultant susceptibility to bacterial and viral infections. Leukocytes show reduced phagocytic and chemotactic functions and defective regulatory T cell responses result in autoimmune manifestations including eczema. Micro thrombocytopenia is a characteristic feature and may occur without eczema or susceptibility to infections in 50% of cases.

Leukocyte adhesion deficiency

Cell surface molecules called integrins serve as adhesion molecules and are essential for leukocyte trafficking. Three types of leukocyte adhesion defects (LAD) have been described. In LAD I, there is impaired expression of beta-2 integrins. LAD II is characterised by mutations in a GDP- fucose transporter. In LAD III activation of all beta integrins are affected due a mutation of Kindlin - 3, which is a cytoplasmic activator of integrins.[9]

Chronic granulomatous disease

Chronic granulomatous disease (CGD) is due to defective phagocyte nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) complex leading to impaired respiratory burst and superoxide formation thereby impaired intracellular killing of catalase positive pathogens.[10] Formation of inflammatory granulomas due to up regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) regulated genes and inflammasome (cytosolic multiprotein oligomers of the innate immune system responsible for the activation of inflammatory responses) activation is a characteristic feature and usually involves the lungs, gastrointestinal and genitourinary tracts. Respiratory burst assays by the nitroblue tetrazolium test (NBT) or the dihydro rhodamine assay (DHR) measures residual superoxide production and is diagnostic. Approximately 80% of CGD is inherited as X - linked disease and is caused by mutations in CYBB which encodes $gp91^{phox}$. Autosomal recessive CGD is caused by mutations in $p22^{phox}$, $p40^{phox}$ $p67^{phox}$ and $p47^{phox}$.

Box 2. Ten warning signs of a possible primary immunodeficiency

Source: These warning signs were developed by the Jeffrey Modell Foundation Medical Advisory Board, www.info4pi.org.

- Four or more new ear infections in a year
- Two or more serious sinus infections in a year
- Two or more months on antibiotics with little effect
- Two or more episodes of pneumonia in a year
- Failure of an infant to gain weight or grow normally
- Recurrent deep skin or organ abscesses
- Persistent oral thrush or cutaneous candidiasis after 1 year of age
- Need for intravenous antibiotics to clear infections
- Two or more deep-seated infections
- A family history of primary immunodeficiency

Mendelian susceptibility to mycobacterial disease (MSMD)

This rare genetic condition is characterized by increased susceptibility to intracellular infections by less virulent bacteria, viruses and fungi. Infections due to environmental mycobacteria, BCG, Salmonella, herpes virus and dimorphic molds occur and M. tuberculosis is also implicated. IL-12/ IFN-γ pathway that involves interactions between mononuclear phagocytes and Th1 cells is important in eliminating intracellular pathogens. In MSMD there is a defect in IFN-γ R1 and IFN-γ R2. Other defects have also been detected like IL-12R β1/β2, IL-12p40, STAT1, Tyrosine kinase 2, IFN regulatory factor 8 and GATA2.[11] BCG adenitis and disseminated BCGiosis are the commonest presentations of MSMD.

Simple clinical clues from the history

The clinical clues to suspect PID are given (Box 2) by the Jeffrey Modell Foundation.

Table I. Infections in common primary immune deficiency disorders

Types of infections	Organisms	Comment
Severe Combined Immune Deficiency		
Pneumonia, diarrhoea, otitis media, pyoderma, sepsis	Parainfluenza 3 virus, adenovirus, RSV, CMV, EBV, varicella-zoster virus, measles virus, P.jirovecii, C. albicans	Wide spectrum of bacterial, fungal, viral and opportunistic pathogens
Vaccine related	MMR, Varicella, BCG	Live vaccines
Abscesses, sinusitis, mastoiditis, pneumonia	S.aureus, C.albicans	Persistent pneumatoceles 'Cold' abscesses
X – Linked agammaglobulinemia		
Pneumonia, diarrhoea, sinusitis, otitis media, meningitis, sepsis	S.pneumoniae, H.influenzae, S.aureus, Candida, Enterovirus, Hepatitis virus, Norovirus	Predominant infections of respiratory and gastrointestinal tracts due to encapsulated organisms. Susceptibility to a narrow spectrum of viruses including enteroviruses may lead to fatal chronic meningo - encephalitis
Hyper IgE syndrome (Autosomal recessive)		
Extensive molluscum contagiosum	CMV, EBV and Cryptosporidia	
Mendelian Susceptibility to Mycobacterial Disease		
	Environmental and atypical mycobacteria, BCG, M. tuberculosis, Salmonella, C. albicans	
MYD88, IRAK - 4 Deficiencies		
Pneumonia, meningitis, sepsis	S. aureus , S. pneumoniae, H. influenzae, P. aeruginosa, Clostridia	Blunted febrile response
Wiskott – Aldrich Syndrome		
Otitis media, pneumonia, meningitis, sepsis.	S. pneumoniae, P. jirovecii, Herpes virus	

MYD88 deficiency – due to mutations in MYD88 gene

IRAK - 4 Deficiencies-interleukin-1 receptor-associated kinase-4 deficiency

Susceptibility to infections

Susceptibility to infections which are severe, persistent, unusual, recurrent (SPUR) is the characteristic of these disorders. Besides, persistent diarrhoea or dermatitis may be the clues in some children. Although recurrent infections are the hallmark of primary immune deficiency disorders, one should rule out secondary immune deficiencies like HIV infection, diabetes mellitus, nephrotic syndrome, exposure to immunosuppressants, malignancies and severe malnutrition upfront. Increased exposure to pathogens at school entry often results in recurrent infections, but they are usually viral upper respiratory infections that are self limiting in nature. Recurrent infections confined to a single site should bring to mind the possibility of an anatomical defect, e.g. recurrent pneumonia in a child with H type of tracheoesopsophageal fistula.

The onset and spectrum of infections give valuable clues for the diagnosis of PIDDs, (Table I). T cell defects have the broadest spectrum involving, bacteria, viruses, fungi and opportunistic pathogens like Candida and Pneumocystis jiroveci.[12]

Onset

Neonatal onset is common in SCID, hyper IgE syndrome and auto inflammatory syndromes like neonatal onset multisystem inflammatory disease (NOMID). B cell defects usually present after 4-6 months of age due to the presence of maternally transmitted antibodies Common variable immunodeficiency (CVID) usually presents in adolescents or adults, although early onset may occur.[13]

Recurrent fever

It is important to remember that fever is not synonymous with infection. While recurrent fever is an important presenting symptom of several PIDDs, it is a characteristic feature in most autoinflammatory syndromes with periodicity being most marked in cyclic neutropenia and periodic fever with aphthous stomatitis, pharyngitis and adenitis (PFAPA). A well-kept fever diary is helpful in diagnosis of autoinflammatory syndromes and is more valuable when accompanied by the documentation of associated features.

Growth and development

Failure to thrive is the hallmark of T cell deficiency[14], although children with B cell defects with concomitant organ impairment e.g. bronchiectasis may also fail to thrive. Gross motor developmental delay is common in children with severe IEIs, but initiation of treatment results in rapid catch up of both growth and development. Short stature with a short lower segment is seen in children with cartilage hair hypoplasia.

Adverse events following immunization

It is important to balance the risk of infection with the efficacy of vaccine and the risk of adverse effects. Adverse events to live vaccines preclude their use in children with several inborn errors of immunity, exceptions are selective IgA deficiency and IgG subclass deficiency. Live bacterial vaccines are contraindicated in children with phagocytic defects like CGD. Specific susceptibility to measles vaccine including life threatening reactions occur in disorders like Signal transducer and activator of transcription (STAT2) deficiency and interferon-alpha receptor-1(IFNa R1) deficiency.[15] Oral polio vaccine can result in life threatening meningoencephalitis in children with B cell defects like XLA and may result in long term excretion with the risk of epidemics with time due to genetic drift in the vaccine virus. Neonates with a family history of SCID should not receive BCG at birth until the disease has been ruled out.

Family history

Most inborn errors of immunity are recessively inherited, either X - linked or autosomal recessive, which explains the male preponderance. A carefully drawn three generation pedigree chart helps ascertain the inheritance pattern. Care should be taken to ask whether the caregivers are the biological parents or not. Fetal loss and deaths of other family members, especially infants should be recorded and the cause of death ascertained by carefully examining old records.

Examination

Syndromic association

Recognition of characteristic syndromes is helpful in making a rapid diagnosis and tailoring management strategies. It is important to remember that all features may not be present and that some may evolve over time. These disorders include ataxia-telangiectasia, autoimmune polyglandular syndrome, cartilage-hair hypoplasia, Wiskott-Aldrich syndrome, Chédiak–Higashi syndrome, DiGeorge syndrome and the hyper-IgE syndrome. Characteristic facial features can be observed in DiGeorge syndrome and AD Hyper IgE syndrome, where it may only be apparent at adolescence.[16]

Table II. Physical signs in common primary immune deficiency disorders

Skin, hair and nails	
Abscesses	CGD, Hyper IgE syndrome
Alopecia	APECED, Omenn syndrome, EDA ID
Eczema	Wiskott - Aldrich syndrome
Erythematous desquamation	Omenn syndrome, IPEX, Netherton syndrome
Hypertrophic scars	LAD
Molluscum contagiosum	HIES AR
Severe pallor	Fanconi anemia, Dyskeratosis congenita, Pearson syndrome
Skin bleeds	Chediak Higashi syndrome
Oculocutaneous albinism	Chediak Higashi syndrome, Griscelli syndrome
Oculocutaneous telangiectasias	Ataxia telangiectasia
Onychomycosis	APECED, mucocutaneous candidiasis, HIES, STAT 1 deficiency, CARD 9 deficiency
Clubbing	XLA with bronchiectasis
Ulcerative lesions with no pus	LAD
Vitiligo	APECED
Oral cavity	
Absent tonsils and non - palpable cervical lymph nodes	XLA, SCID
Gingivitis	LAD
Oral ulcers	CGD, HIGM, HIDS
Persistent / recurrent thrush	SCID, HIGM, APECED, CMC, DiGeorge syndrome
Bifid uvula / submucous cleft palate	DiGeorge syndrome
Extremities	
Arthritis	XLA, AIS
Fractures	HIES AD
Joint laxity	HIES AD, cartilage hair hypoplasia
Short limbs	Cartilage hair hypoplasia

AIS - Autoinflammatory syndrome, APECED - Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, CARD 9 deficiency - mutations in caspase recruitment domain family member 9 gene, CGD - Chronic Granulomatous Disease, CMC - Chronic mucocutaneous candidiasis,EDA ID - Anhidrotic ectodermal dysplasia with immune deficiency, HIDS - Hyper IgD syndrome, HIES - Hyper IgE syndrome, HIGM - Hyper IgM syndrome, IPEX- Immune deficiency, polyendocrinopathy X – linked, LAD – Leukocyte Adhesion Deficiency, XLA – X linked agammaglobulinemia.

Common clues for a PIDD on physical examination

The physical examination often yields valuable clues for the diagnosis of a PIDD and enables prioritization of investigations (Table II). Disorders that are associated with lymphoproliferation with prominent lymphadenopathy and splenomegaly include autoimmune lymphoproliferative syndrome (ALPS), X-linked lymphoproliferative syndrome (XLP), Immune dysregulation, polyendocrinopathy,

Table III. Autoimmune manifestations in primary immune deficiency disorders

Dermatological	
Alopecia	CVID, IPEX, Omenn syndrome
Eczema	WAS
Psoriasis	CVID, IPEX
Vitiligo	CVID, IPEX
Endocrine	
Thyroiditis	APECED, IPEX, XLA, AT, HIES AR, CTLA4, LRBA deficiency
Type 1 Diabetes mellitus	APECED, IPEX, XLA, CTLA4, LRBA deficiency
Gastrointestinal	
Celiac / celiac – like disease	CVID, APECED
Enteropathy	IPEX, CVID, HIES AR
Autoimmune hepatitis	ALPS
IBD	WAS, HIGM, CVID, CGD, APECED
Sclerosing cholangitis	ALPS
Hematological	
AIHA	ALPS, WAS, HIGM, DGS, XLA, Omenn syndrome, HIES AR
Evans syndrome	ALPS, WAS, DGS
Neutropenia	ALPS, WAS, XLA
Thrombocytopenia	ALPS, WAS, HIGM, DGS, CGD, XLA, Omenn syndrome
Ophthalmological	
Uveitis	Blau syndrome, ALPS, CGD, LRBA deficiency, WAS, hyper IgM syndrome
Optic neuritis	ALPS
Pulmonary	
Granulomatous–lymphocytic interstitial lung disease (GLILD)	CVID, LRBA deficiency, CTLA4 deficiency
Renal	
IgA nephropathy	WAS
Rheumatological	
JIA	CGD, XLA, IgA deficiency
Systemic	
SLE	CGD, C1r, C1s, C4, C2 deficiency
Vasculitis	AT, ALPS, DiGeorge, HIES AR

AT – Ataxia Telangiectasia, LRBA deficiency - Lipopolysaccharide (LPS)-responsive and beige-like anchor protein deficiency, CTLA4 deficiency - Cytotoxic T-lymphocyte-associated protein-4 deficiency, CVID – Common Variable Immune Deficiency, WAS - Wiskott Aldrich syndrome.

enteropathy, X-linked (IPEX), Cytotoxic T-lymphocyte-associated protein-4 deficiency (CTLA4), Lipopolysaccharide (LPS)-responsive and beige-like anchor (LRBA)protein deficiency, Activated phosphatidylinositol 3-kinase-δ (PI3K δ) Syndrome.[17]

Autoimmunity

Autoimmune manifestations are most common among patients with inborn errors of immunity with immune dysregulation and include multisystem manifestations (Table III). APECED, ALPS, IPEX, CTLA4 deficiency, LRBA deficiency and PI3Kδ Syndrome are common examples of diseases with prominent autoimmune manifestations.[18] Autoimmune manifestations occur earlier in T cell immune deficiency than in B cell defects.

Autoinflammation

These disorders involve unusual inflammation mediated by aberrant activation of the innate immune system and autoreactive T-cells or autoantibodies are characteristically absent. Recurrent or periodic fever with inflammation of the skin, mucosae and / or synovium occur in association with elevated inflammatory markers interspersed with asymptomatic periods and absence of inflammatory markers (Table IV). The flares may last less than a day as in familial cold autoinflammatory syndrome (FCAS) or may be prolonged over weeks as in tumour necrosis factor receptor associated periodic syndrome (TRAPS). Oral ulcers, lymphadenitis, rash, arthritis and gastrointestinal symptoms are common. Long term morbidity may result from amyloidosis and associated complications or deafness.[19] Poor awareness among clinicians often results in delayed diagnosis and burdensome therapies and surgical procedures.

Risk of malignancy

The risk of developing a malignancy is 4-25% in patients with PIDDs and lymphomas (both Non - Hodgkin's

Table IV. Clinical characteristics of the common autoinflammatory syndromes

Clinical features	FMF	FCAS	MWS	NOMID	HIDS	TRAPS	PFAPA
Fever < 1 day	-	+	+	-	-	-	-
Fever < 7 days	+	-	-	-	+		+
Prolonged fever	-	-	-	+	-	+	-
Regular periodicity	-	-	-	-	-	-	+
GI symptoms	+	-	-	-	+	+	rare
Conjunctivitis	-	+	+		-	+	-
Lymphadenitis	+	-	-	+	+	+	+
Peritonitis	+	-	-	-		+	
Aphthous ulcers	-	+	+	-	+	+	+
Arthritis	+	+	+	+	+	+	-
Rash	Erysipelas-like	Cold-induced urticaria	Urticarial	Urticarial	Maculo-papular/ urticarial	Migratory	-
Splenomegaly	+	-	-	+	+	+	-
Improving with age	-	-	-	-	+	-	+
Amyloidosis	+	-	+	+	Rare	+	-
Hearing loss	-	-	+	+	-	-	-

FCAS - Familial cold autoinflammatory syndrome, FMF - Familial Mediterranean Fever, HIDS - Hyperimmunoglobulin D syndrome, MWS - Muckle - Wells syndrome, NOMID - Neonatal - onset multisystem inflammatory disease, PFAPA - Periodic fever with aphthous stomatitis, pharyngitis and cervical adenitis syndrome, TRAPS - TNF-Receptor Associated Periodic Syndrome.

Table V. Risk of malignancy in primary immune deficiency disorders

Primary immune deficiency disorder	Risk of malignancy
Ataxia telangiectasia	Non - Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, T cell ALL, breast cancer
Autoimmune lymphoproliferative syndrome	Lymphoma
Common variable immunodeficiency	Gastric carcinoma, thymic cancer, NHL, Hodgkin's lymphoma
Hyper - IgE syndrome AR	Squamous cell carcinoma, lymphoma
X – linked Hyper - IgM syndrome	Cholangiocarcinoma, neuro - ectodermal tumours
Nijmegen breakage syndrome	Lymphoma, acute leukemia
Schwachman – Diamond syndrome	Lymphoma, leukemia
Severe congenital neutropenia	Leukemia
Wiskott - Aldrich syndrome	Lymphoma, leukemia, cerebellar astrocytoma, Kaposi sarcoma
X-linked lymphoproliferative disease	Lymphoma

and Hodgkin's) occur most often (Table V). Malignancies in these patients are often diagnosed at a late stage and hence have worse outcomes.[20] Patients with ataxia telangiectasia and common variable immune deficiency have the highest risk of developing cancer.

To conclude, primary immune deficiency disorders are not uncommon and require a high index of suspicion for diagnosis. Susceptibility to severe, persistent, unusual, recurrent and difficult to treat infections is the commonest clue for suspecting PIDD. However, autoimmune, allergic or autoinflammatory features may be predominant. An enhanced risk of malignant disorders is also a feature in several of these disorders. Outcomes are dramatically improved with early diagnosis and optimal management, for which sensitization and enhancing awareness among pediatricians is crucial.

Points to Remember

- ***A high index of suspicion is essential to diagnose primary immune deficiency disorders (PIDDs).***
- ***Infections of unusual severity, frequency, etiology and suboptimal response to treatment are the hallmark of these disorders.***
- ***Apart from susceptibility to infections, autoimmune, allergic and autoinflammatory features occur as well as early onset malignancies.***
- ***Important clues from the history include age at onset, types of infections, adverse events following immunization and the family history.***
- ***Examination often reveals valuable clues including syndromic features, paucity or proliferation of lymphoid tissue, other systemic manifestations and failure to thrive.***

Acknowledgements

The authors wish to thank SERB and CSIR - IGIB, Delhi for their support.

References

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 2020; 40(1):24-64.
2. Bousfiha AA, Jeddane L, Ailal F, Benhsaien I, Mahlaoui N, Casanova JL, et al. Primary immunodeficiency diseases worldwide: more common than generally thought. J Clin Immunol 2013; 33(1):1-7.
3. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy Asthma Clin Immunol 2018; 14(Suppl2):49.
4. Tasher D, Dalal I. The genetic basis of severe combined immunodeficiency and its variants. Appl Clin Genet 2012; 5:67-80.
5. Kumrah R, Vignesh P, Patra P, Singh A, Anjani G, Saini P, et al. Genetics of severe combined immunodeficiency. Genes Dis 2020; 7(1):52-61.
6. Amado MC. Primary Immunodeficiency Update and Newborn Screening. Mo Med2011; 108(5):350-353.
7. Suri D, Rawat A, Singh S. X-linked Agammaglobulinemia. Indian J Pediatr 2016; 83(4):331-337.

8. Blundell MP, Worth A, Bouma G, Thrasher AJ. The Wiskott-Aldrich Syndrome: The Actin Cytoskeleton and Immune Cell Function. Dis Markers 2010; 29 (3-4):157-175.
9. Das J, Sharma A, Jindal A, Aggarwal V, Rawat A. Leukocyte adhesion defect: Where do we stand circa 2019? Genes Dis 2020; 7(1):107-114.
10. Arnold DE, Heimall JR. A Review of Chronic Granulomatous Disease. Adv Ther 2017; 34(12):2543-2557.
11. Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinical features of inborn errors of IFN-γ immunity. Semin Immunol 2014; 26(6):454-470.
12. Gathmann B, Mahlaoui N, Gérard L, Oksenhendler E, Warnatz K, Schulze I, et al. Clinical picture and treatment of 2212 patients with common variable immunodeficiency. J Allergy Clin Immunol 2014; 134(1):116-126.
13. Griffith LM, Cowan MJ, Notarangelo LD, Puck JM, Buckley RH, Candotti F, et al. Improving cellular therapy for primary immune deficiency diseases: recognition, diagnosis, and management. J Allergy Clin Immunol 2009; 124(6):1152-1160.e12.
14. Barron MA, Makhija M, Hagen LE, Pencharz P, Grunebaum E, Roifman CM. Increased resting energy expenditure is associated with failure to thrive in infants with severe combined immunodeficiency. J Pediatr 2011; 159(4):628-632.e1.
15. Hambleton S, Goodbourn S, Young DF, Dickinson P, Mohamad SMB, Valappil M, et al. $STAT_2$ deficiency and susceptibility to viral illness in humans. Proc Natl Acad Sci U S A. 2013; 110(8):3053-3058.
16. Govindaraj GM, Riyaz A, Krishnan C, Scaria. V. Rapid Transition of Facial Features from Early to Mid - Adolescence in Autosomal Dominant Hyper IgE Syndrome with a $STAT_3$ Variation. Indian J Pediatr 2018; 85(7): 595-596.
17. Natkunam Y, Gratzinger D, Chadburn A, Goodlad JR, Chan JKC, Said J, et al. Immunodeficiency-associated lymphoproliferative disorders: time for reappraisal? Blood 2018; 132(18):1871-1878.
18. Carneiro-Sampaio M, Coutinho A. Early-onset autoimmune disease as a manifestation of primary immunodeficiency. Front Immunol 2015; 6:185.
19. DeSanctis S, Nozzi M, Del Torto MD, Scardapane A, Gaspari S, de Michele G, et al. Autoinflammatory syndromes: diagnosis and management. Ital J Pediatr 2010; 36:57.
20. Mayor PC, Eng KH, Singel KL, Abrams SI, Odunsi K, Moysich KB, et al. Cancer in primary immunodeficiency diseases: Cancer incidence in the United States Immune Deficiency Network Registry. J Allergy Clin Immunol 2018; 141(3):1028-1035.

CLIPPINGS

Rapid Point-of-Care Multiplex Immunochromatographic Assay for the Diagnosis of Enteric Fever.

There is a critical need for an improved rapid diagnostic for enteric fever. IgA responses targeting *Salmonella enterica* serovarTyphihemolysin E (HlyE) and lipopolysaccharide (LPS) are able to discriminate patients with acute typhoid from healthy controls in areas where enteric fever is endemic (healthy endemic controls) and from patients with other bacterial infections. Data demonstrating that IgA antibody responses against these antigens also work well for identifying patients with acute *S.* Paratyphi A infection. To develop a test for acute enteric fever detection, we have adapted a point-of-care immunechromatographic dual-path platform technology (DPP), which improves on the traditional lateral flow technology by using separate sample and conjugate paths and a compact, portable reader, resulting in diagnostics with higher sensitivity and multiplexing abilities.

In this analysis, we have compared our standard enzyme-linked immunosorbent assay (ELISA) method to the DPP method in detecting acute phase plasma/serum anti-HlyE and anti-LPS IgA antibodies in a cohort of patients with culture-confirmed *S.* Typhi (n = 30) and Paratyphi A infection (n = 20), healthy endemic controls (n = 25), and febrile endemic controls (n = 25). It was found that the DPP measurements highly correlated with ELISA results, and both antigens had an area under the curve (AUC) of 0.98 (sensitivity of 92%, specificity of 94%) with all controls and an AUC of 0.98 (sensitivity of 90%, specificity of 96%) with febrile endemic controls. Our results suggest that the point-of-care DPP Typhoid System has high diagnostic accuracy for the rapid detection of enteric fever and warrants further evaluation.

Kumar S, Nodoushani A, Khanam F, DeCruz AT, Lambotte P, Scott R, et al. Evaluation of a Rapid Point-of-Care Multiplex Immunochromatographic Assay for the Diagnosis of Enteric Fever.mSphere.2020 Jun 1128/ mSphere.00253-2010;5(3):e00253-20. doi:10..PMID: 32522777.

IMMUNOLOGY

LABORATORY CLUES TO PRIMARY IMMUNODEFICIENCY DISORDERS

***Sagar Bhattad**
****Rachna Shanbhag Mohite**

Abstract: *Primary immunodeficiency disorders are a large group of heterogeneous diseases, which result from defects in the immune system. These defects can either be in the innate or adaptive immunity. As per the latest classification published by the International Union of Immunological Societies expert committee on Inborn Errors of Immunity, around 430 primary immunodeficiency disorders have been recognized and the list is expanding. One in 1000 individuals suffer from a primary immunodeficiency disorder and these diseases are by no means, rare. Yet many patients remain undiagnosed, due to lack of awareness of these conditions. This article highlights the importance of routine tests like complete blood counts and serum immunoglobulin assay in diagnosing patients with these disorders. Along with case-based discussion, simple algorithms have been provided, that can guide a clinician in making a timely diagnosis.*

Keywords: *Primary immune deficiency diseases, Inborn errors of immunity, Laboratory tests.*

Primary immunodeficiency disorders (PID) are a large heterogeneous group of diseases, which result from defects in the immune system development and/or function. These diseases are now better known as inborn errors of immunity (IEI). PIDs can be broadly classified as disorders of innate immunity (e.g. phagocyte and complement disorders) and adaptive immunity (i.e. T cell, B-cell or combined immunodeficiencies). The term PID now also includes increasing number of syndromes that are associated with autoimmunity and immune dysregulation.[1]

The International Union of Immunological Societies (IUIS) expert committee (EC) on Inborn Errors of Immunity (IEI) regularly publish an updated classification of IEI every two years. This classification provides valuable information regarding the clinical features, disease-causing genotypes and immunological defects in various PIDs. Every year, new PIDs are added to the classification, a reflection of the advancements noted in molecular genetics and sequencing technologies. The 2019 International Union of Immunological Societies (IUIS) classification now comprises of 406 distinct disorders with 430 different gene defects. The classification serves as a diagnostic tool for clinicians at the bedside.[2,3]

As per Jeffery Modell Immune Deficiency Foundation USA, one in 1000 individuals suffer from a PID.[4] Based on this estimation, there must be thousands of such patients in the Indian subcontinent. Unfortunately, most of these patients remain undiagnosed for years. Being genetic diseases, they afflict several children in a given family and the diagnosis is missed unless there is a focused evaluation of all family members by a single physician.

Simple tests provide valuable clues to the diagnosis

Immune deficiencies are a heterogeneous group of diseases with variable clinical presentations. Clinicians seldom attempt to diagnose these conditions since the general perception is that, advanced immunological tests must be at hand to diagnose any immunological disorder. On the contrary, most PIDs can be suspected by analyzing routinely available tests. The eyes see only what the mind knows - once a clinician is geared up to pick up these conditions, diagnosing PIDs becomes an easy task. Routine tests like complete blood counts provide valuable clues to underlying PIDs. Tests like serum immunoglobulin assays are now readily available at most places in the country and can clinch the diagnosis in a vast majority of patients. These tests are cost effective and must be used more often in clinical practice.

In this article, an attempt is made to highlight how every Pediatrician can diagnose PIDs using a practical approach by showing (Fig.1) and by illustrative case scenarios.

* Consultant
** Fellow,
Division of Pediatric Immunology and Rheumatology,
Department of Pediatrics,
Aster CMI Hospital, Bangalore.
email: drsagarbhattad@gmail.com

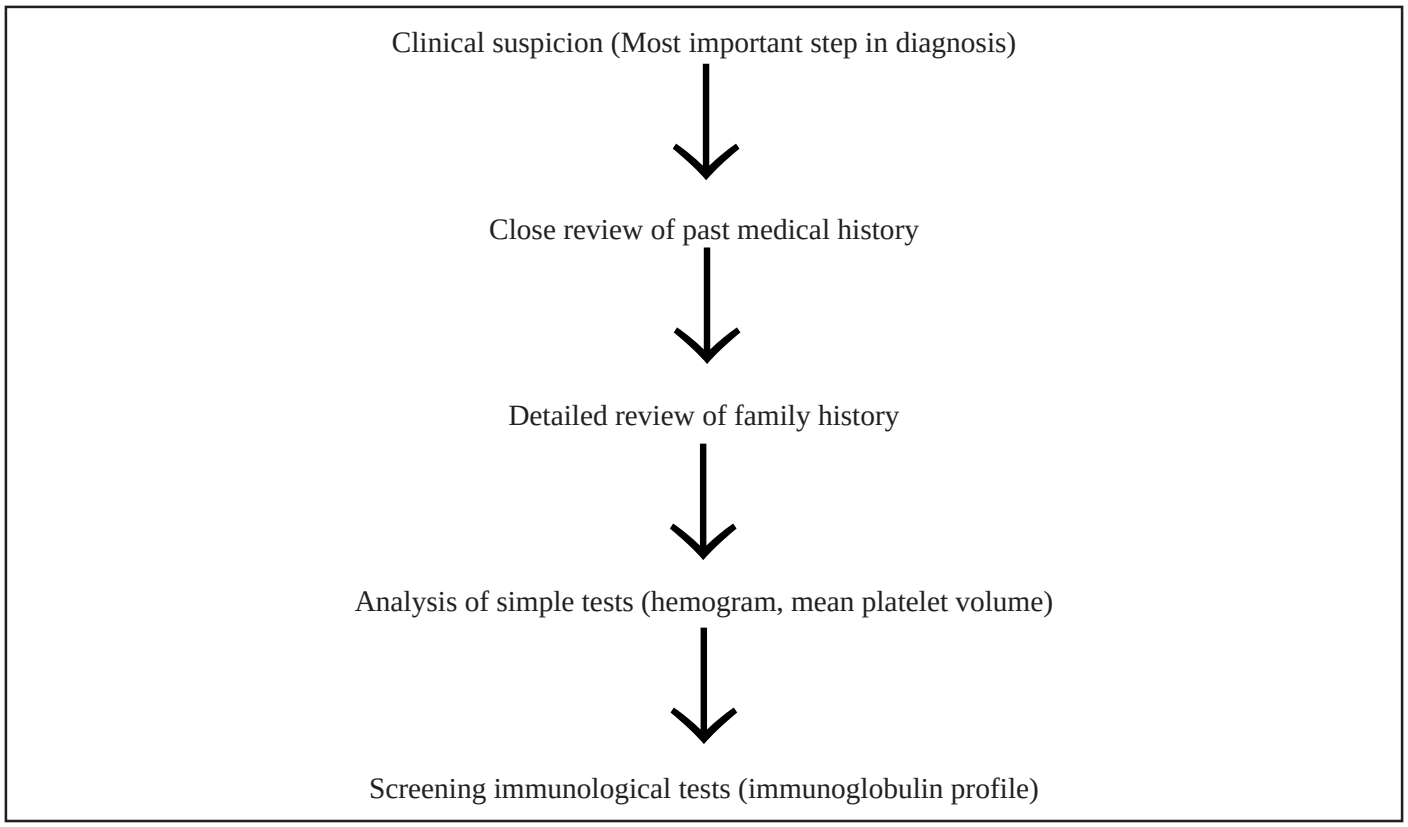


Fig.1. Approach to the diagnosis of PIDs

Clues from the hemogram

A complete blood count (CBC) with differential is the most valuable test in diagnosing a variety of PIDs.

Lymphopenia: The most common diagnosis offered to sick febrile children in clinical setting is sepsis. The presence of neutrophilia in this setting often makes one think of sepsis and commonly no focus is laid on lymphocyte counts.

Whereas a CBC indicating persistent lymphopenia must raise a suspicion of T cell defect. Physiologically, lymphocytic predominance is noted in infancy.[5] An absolute lymphocyte count of less than 3000/mm^3 in an infant can be used as a cut off for suspecting a T cell disorder.[6]

As lymphocyte numbers are age dependent, knowledge of normal values for absolute lymphocyte counts at various ages is crucial. T-cell disorders like severe combined immune deficiency (SCID) present with recurrent infections and persistent lymphopenia.[7]

Case 1

A 4-month-old female infant presented with recurrent episodes of oral thrush from first month of life. She had been treated with topical antifungals and a course of oral fluconazole had also been given. She had an episode of pneumonia at 3 months of age, which responded to intravenous antibiotics. In view of persistent thrush during this episode she was referred to the pediatric immunology services.

Investigations done -

HIV rapid test: Negative

CBC: Hemoglobin (Hb)-8.8mg/dL, Total white cell counts (TC)-10,000/mm^3 (N_{84} L_6 M_6 E_4), Platelet counts (PC)-1,43,000/mm^3. Absolute neutrophil count (ANC): 8400/mm^3. Absolute lymphocyte count (ALC): 600/mm^3.

Chest Xray: showed absence of thymic shadow.

On reviewing records, lymphopenia was noted in all the hemograms. In view of persistent lymphopenia in a young infant, severe T cell defect like SCID was suspected and further immunological work up was carried out. The findings were IgG <120 mg/dL (176-601), IgA <16 mg/dL (4.4-84) and IgM <15mg/dL (17-105). Lymphocyte subsets: T-1% B-98% NK-1% (T-B+NK-).

With absent T cells and hypogammaglobulinemia in an infant, a diagnosis of SCID was made and the child later underwent a successful bone marrow transplant.

Message: Persistent lymphopenia in a young child - **THINK OF SCID.**

Neutropenia: Neutropenia is defined as an ANC less than 1500/mm^3 in children more than 1 year of age and ANC less than 2000/mm^3 in children less than 1 year of age.[8] An approach to neutropenia is discussed in Fig.2. Severe congenital neutropenia is a rare primary immunodeficiency disease with a bone marrow maturation arrest of granulocytic differentiation at the promyelocyte-myelocyte stage.[9,10] These patients are at an increased risk for severe fatal infections and often die in the first 2 years of life, unless treated appropriately.

Case 2

A 5-month-old girl child, born of second-degree consanguinity, presented with draining ears from second month of life. She was hospitalized with severe pneumonia.

Family history: She was first born baby. No history of sibling death.

Investigations

CBC: Hb – 8.5 g/dL, TC - 7300 /mm^3 (N8 L80 M6 E5), PC - 5, 23,000 /mm^3

ANC: 584 /mm^3

Previous records were reviewed -

CBC: Hb - 9 g/dL, TC - 5200 /mm^3 (N4 L75 M15 E5), PC - 2, 36,000 /mm^3

ANC: 208 /mm^3

CBC: Hb - 9.2 g/dL, TC - 6500 /mm3 (N7 L78 M12 E3), PC - 6, 39,000 /mm^3

ANC: 455 /mm^3

Neutrophils were persistently reduced in number. Intermittent monocytosis was noted. Bone marrow biopsy showed maturation arrest in the myeloid lineage (only precursors in the myeloid lineage were seen). Erythroid and megakaryocytic lineage were normal.

Genetic studies: Pathogenic mutation in ELANE gene.

Diagnosis: Severe congenital neutropenia

Message: Persistent severe neutropenia in infants - Think of severe congenital neutropenia**.**

Cyclic neutropenia is a rare blood disorder in which there is recurrent episodes of abnormally low levels of neutrophils. These children present with profound neutropenia for 3-6 days with an average cycle lasting 21 days.[11,12] There is cyclic haematopoiesis with monocytosis during the phase of neutropenia. During the nadir of neutropenia, patients are prone to severe infections involving the mucosal sites, such as oral cavity, upper respiratory tract or rectal mucosa.[13] These infections can be life threatening in some patients if not diagnosed and treated promptly.

Case 3

An eight year-old girl presented with recurrent episodes of oral ulcers from 4 year of age. These ulcers would last for 4-5 days and recur almost monthly. She had been hospitalized twice for pneumonia in the past 2 years. She had received oral antibiotics for otitis media, a year ago.

She had undergone multiple blood tests and when the records were analysed, the following were noted.

CBC: Hb - 10.2 g/dL, TC - 5200 /mm^3 (N5 L75 M15 E5), PC - 4, 26,000 /mm^3

ANC: 260 /mm^3

CBC: Hb - 11 g/dL, TC - 8200 /mm^3 (N65 L25 M5 E5), PC - 2, 39,000 /mm^3

ANC: 5330 /mm^3

CBC: Hb - 9.2 g/dL, TC - 6500 /mm^3 (N7 L78 M12 E3), PC - 5, 38,000 /mm^3

ANC: 455 /mm^3

CBC: Hb - 9 g/dL, TC - 7500 /mm^3 (N78 L12 M7 E3), PC - 6, 39,000 /mm^3

ANC: 5850 /mm^3

This child had intermittent neutropenia (and monocytosis too).

She was suspected to have cyclic neutropenia and serial CBCs were asked for. In order to diagnose cyclic neutropenia, CBC must be performed twice a week for 6 weeks. Details have been tabulated (Table I).

Cyclic neutropenia was diagnosed and genetic testing showed pathogenic mutation in ELANE gene.

Message: In children presenting with recurrent fevers, oral ulcers, draining ears, one must closely look at ANC and periodic monitoring of ANC can provide a clue to the underlying cyclic neutropenia. These patients do well with Granulocyte colony-stimulating factor (G-CSF) therapy.

Neutrophilia

Neutrophil counts in a CBC report are used routinely as a part of the sepsis screen. Neutrophilia can be defined as ANC value greater than 2 standard deviations above the mean value for the age-defined population.[14,15] The most

Table I. Serial CBCs in a child with suspected cyclic neutropenia

Date	Hb (g/dL)	TC (per mm^3)	DC	PC (per mm^3)	ANC (per mm^3)
1st June	10	7600	N60 L30 M6 E4	4,50,000	4560
4th June	10.2	6500	N45 L40 M8 E7	3,00,000	2925
7th June	9.5	4100	N5 L 65 M25 E5	2,30,000	205
10th June	9.8	4200	N10 L60 M20 E7	2,00,000	420
13th June	10	6700	N45 L50 M3 E2	2,23,000	3015
16th June	10.3	7000	N67 L25 M6 E2	3,50,000	4690
19th June	10.5	8700	N70 L22 M5 E3	3,40,000	6090
22nd June	10	7700	N58 L25 M9 E6 B2	2,45,000	4466
25th June	10.2	8340	N35 L55 M5 E5	2,34,000	2905
28th June	9.4	4000	N3 L60 M27 E7 B3	1,65,000	120
1st July	10.3	4500	N10 L62 M20 E6 B2	1,87,000	450
4th July	10.2	8000	N68 L24 M6 E2	2,89,000	5440

frequent causes for neutrophilia in neonates include infection, antenatal/postnatal treatment with corticosteroids etc. But persistence of neutrophilia in neonates and infants even in the absence of infection must raise the suspicion for leukocyte adhesion deficiency.[16]

Case 4

A 7-month-old female infant presented with chronic diarrhea for the past 4 months and had developed perianal ulcers. She had marked neutrophilia and was thought to have sepsis and given multiple courses of antibiotics,

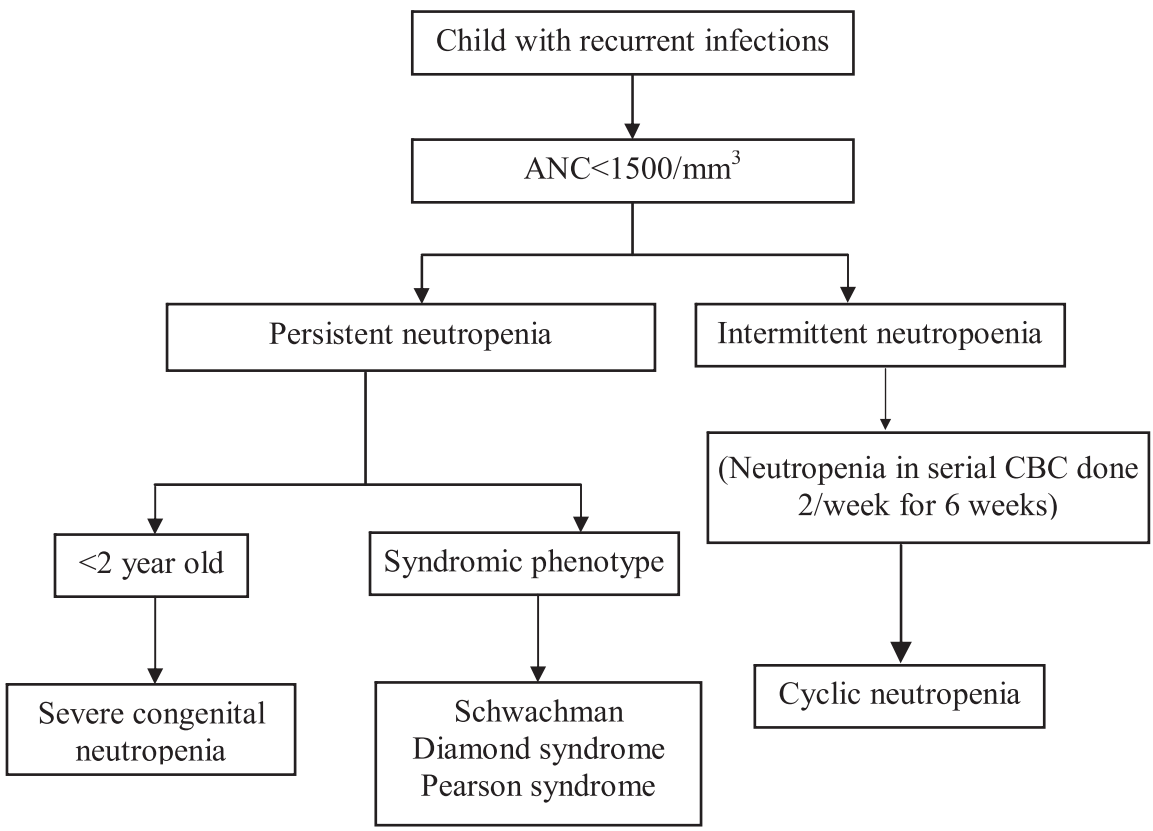


Fig.2. Approach to neutropenia

without any relief. She was referred to Immunology department with a suspicion of PID. Her previous records were analyzed. There is also history of delayed separation of umbilical cord.

CBC: Hb - 10.2 g/dL, TC - 95,000 / mm^3 (N85 L10 M4 E1), PC - 5,36,000/ mm^3

ANC: 80750/mm3

CBC: Hb - 11 g/dL, TC - 108,200 / mm^3 (N82 L12 M5 E1), PC - 1, 39,000 / mm^3

ANC: 88,560 / mm^3

CBC: Hb - 9.2 g/dL, TC - 84,500 / mm^3 (N87 L8 M3 E2), PC - 4, 65,000 / mm^3

ANC: 73,515 / mm^3

CBC: Hb - 9 g/dL, TC - 67,500 / mm^3 (N82 L10 M7 E1), PC - 6, 29, 000 / mm^3

ANC: 55,350 / mm^3

She had persistent neutrophilic leukocytosis and the counts remained high even when the child was clinically well. A clinician must think beyond sepsis in such a setting.

Immunological work up

CD18 expression on neutrophils was studied by flow cytometry and was remarkably reduced.

CD18 - 0.1% (normal - 99%).

Genetic test: reported pathogenic mutation ITGB2 gene.

Diagnosis: Leukocyte adhesion deficiency type 1.

(Note: A diagnosis of LAD type I can be established based on reduced CD18 expression on neutrophils on flow cytometry. Genetic test is recommended for confirmation and offering genetic counselling.)

Message: Persistent neutrophilic leukocytosis must raise suspicion of leukocyte adhesion deficiency.

Thrombocytopenia

Thrombocytopenia is not an uncommon occurrence in clinical practice and the list of conditions known to cause thrombocytopenia is too long. However, presence of persistent thrombocytopenia must make one think of PID.

Patients with Wiskott Aldrich Syndrome (WAS) present with thrombocytopenia, eczema, recurrent infections and immunodeficiency.[17] Interestingly these patients have small platelets (micro thrombocytopenia) and this is probably the only condition in medicine that does so (X-linked thrombocytopenia, a milder variant of WAS can also present with micro thrombocytopenia). It is prudent to look at the mean platelet volume in every child presenting with persistent thrombocytopenia.

Case 5

A 4-year-old boy presented with repeated pyoderma from the first year of life. He had severe eczema and was being treated with topical steroids by the dermatologist. He was hospitalized with an episode of pneumonia.

Investigations

CBC: Hb-10g/dL, TC-13,000/mm^3 (N_{65} L_{20} M_6 E_4), PC-50,000/mm^3.

HIV rapid card test: Negative

Mean platelet volume (MPV): 5ft (7-11) suggestive of microthrombocytopenia.

Diagnosis: Wiskott-Aldrich syndrome.

The presence of a triad of thrombocytopenia, eczema and immune deficiency confirms the diagnosis of Wiskott Aldrich syndrome (WAS). However, all patients with WAS do not present with the triad. Pediatricians must think of WAS in every boy with persistent thrombocytopenia even in the absence of eczema or infections.

Genetic testing: Mutation in Wiskott Aldrich syndrome protein (WASP) gene

Treatment offered: Intravenous immunoglobulin infusions followed by bone marrow transplant.

Message: Boy with persistent thrombocytopenia - think of Wiskott-Aldrich syndrome.

These cases aptly highlight the importance of a hemogram in the diagnosis of PIDs. One must spend more time in analyzing the differential white cell counts and the value of this exercise in making a timely diagnosis of PID cannot be overemphasized.

Serum immunoglobulin assay and its utility in the diagnosis of PIDs

Immunoglobulins/antibodies are proteins produced by B cells and play a pivotal role in defending the body against infections. Activated B cells secrete one of the four major classes of antibody: IgG, IgM, IgA, and IgE. There are also immunoglobulin subclasses, including 4 subclasses of IgG (IgG1, IgG2, IgG3 and IgG4) and 2 subclasses of IgA (IgA1 and IgA2). Immunoglobulins in each subclass have different biologic roles, for example- IgG2 is important in handling encapsulated bacteria. At birth, newborns are

susceptible to infections because of immaturity of the immune system. During the first few months of life, they have some immunity from transplacentally transferred maternal IgG antibodies and they start producing IgG antibodies after 3-6 months of age.[18] Other antibodies (IgA, IgM, IgE) mature slowly and take several years to reach adult values. It is thereby important to note that immunoglobulin levels are age specific and should be interpreted using the age specific normative data. Children presenting with deficiency in immunoglobulin production are at increased risk of sinopulmonary infections. These group of disorders are called humoral immune deficiencies. A simple algorithm highlighting the use of immunoglobulin assays in clinical practice is presented in Fig.3.

Case 6

A 3.5-year-old male child was referred with a history of recurrent chest infections. He had 4 episodes of pneumonia, requiring admission and intravenous antibiotics (first episode at 6 months of life). He also had 3 episodes of ear discharge. He had been previously evaluated for recurrent pneumonia.

Investigations done

Echocardiography - normal (excluded congenital heart disease as a possibility)

CT chest - normal (ruled out congenital lung anomaly)

Sweat chloride test - normal (cystic fibrosis unlikely)

Gastroesophageal reflux scan- normal (ruled out the possibility of aspiration syndromes)

In view of recurrent sinopulmonary infections, PID was suspected and immunological tests were asked for:

IgG <120 mg/dL (345-1236), IgA <20 mg/dL (14-159) and IgM <30mg/dL (43-207)

Lymphocyte subsets: B cell counts: 1% (10-15%) {lymphocyte phenotyping is done using flow cytometry}

Genetic testing: Mutation in Bruton tyrosine kinase (BTK) gene.

Diagnosis: X-linked agammaglobulinemia (XLA).

Message: Serum immunoglobulins must be tested in children with recurrent pneumonia.

Case 7

A 35-year-old gentleman was unwell from the age of 10, and had been treated for recurrent sinusitis with several courses of antibiotics. For the past three years, he was hospitalized five times for pneumonia. Extensive evaluation including chest imaging (CT chest) was not-rewarding. He was referred to the Immunology services.

Immunological evaluation showed: IgG: 260 mg/dL (639 - 1349), IgA: <25 mg/dL (70 - 312)

And IgM: 30 mg/dL (56 - 352)

Lymphocyte subsets:CD3:70% (50-75), CD19:15% (10-15), CD56:12% (5-10)

Diagnosis: Common variable immune deficiency (CVID).

Message: In adolescents and adults presenting with recurrent sino-pulmonary infections and/or chronic diarrhea - one must think of CVID.

Case 8

A 3-year-old boy presented with the third episode of severe pneumonia. He had been ventilated for 10 days during the first episode at the age of 6 months. After extensive evaluation (echocardiography, CT chest, cystic fibrosis work up), he was referred to immunology department.

We looked at the immunoglobulin profile: IgG: 27 mg/dL (345 - 1236), IgA: <20 mg/dL (14 - 159), IgM : >530 mg/dL (43 - 207).

B cell counts: Normal.

He had markedly elevated IgM levels, while IgG and IgA were undetectable.

Diagnosis: Hyper-IgM syndrome.

Genetic testing: Mutation in CD40L gene, confirming hyper-IgM syndrome type-1.

Message: Children with hyper IgM syndrome can present with recurrent infections and estimation of serum immunoglobulins provides timely diagnosis.

Case 9

A 16-year old adolescent presented with high grade fever for 3 weeks. He also complained of severe backache. MRI spine showed vertebral osteomyelitis affecting lumbosacral region and Staphylococcus aureus was isolated on blood culture. He was treated with a prolonged course of intravenous antibiotics and made a gradual recovery.

Past history was significant for severe pneumonia and empyema at the age of 5 years requiring hospitalisation. Repeated skin infections and eczema were noted from a

young age. He had pathological fracture of both bones of right forearm at the age of 10, following a trivial fall.

Family history: Non-consanguineous parentage. Father had been treated for severe pneumonia twice in the past, each episode requiring hospitalization for 7-10 days. He also had severe eczema from childhood and coarse facies.

Investigations in the index case

CBC: Hb - 10g/dL, TC - 14,300/mm^3 (N69 L15 M6 E10), PC - 5, 28,000/ mm^3

AEC: 1430 / mm^3

IgG: 1220 mg/dL (639-1349), IgA:156 mg/dl (70-312), IgM:130 mg/dL (56-352)

IgE - 3460 IU (<60)

Father was also evaluated: IgG:1380 mg/dL (639-1349), IgA:160 mg/dL (639-1349), IgM:176 mg/dL (56-352).

IgE - 2345 IU (<60)

Since both the father and the child were affected, autosomal dominant (AD) pattern of inheritance was suspected and genetic studies showed heterozygous pathogenic mutation in signal transducer and activator of transcription 3 (STAT3) gene in the child and the father.

Diagnosis: AD hyper-IgE syndrome

Message: Recurrent infections and eczema - Hyper IgE syndrome is a differential.

Case 10

A 10-year-old girl presented with recurrent rhinosinusitis and repeated episodes of pneumonia. She had been hospitalized on at least 3 occasions so far. Blood culture grew Streptococcus pneumoniae on one occasion. Extensive evaluation for the cause of repeated infections (including CT chest, echocardiography etc) was non-rewarding.

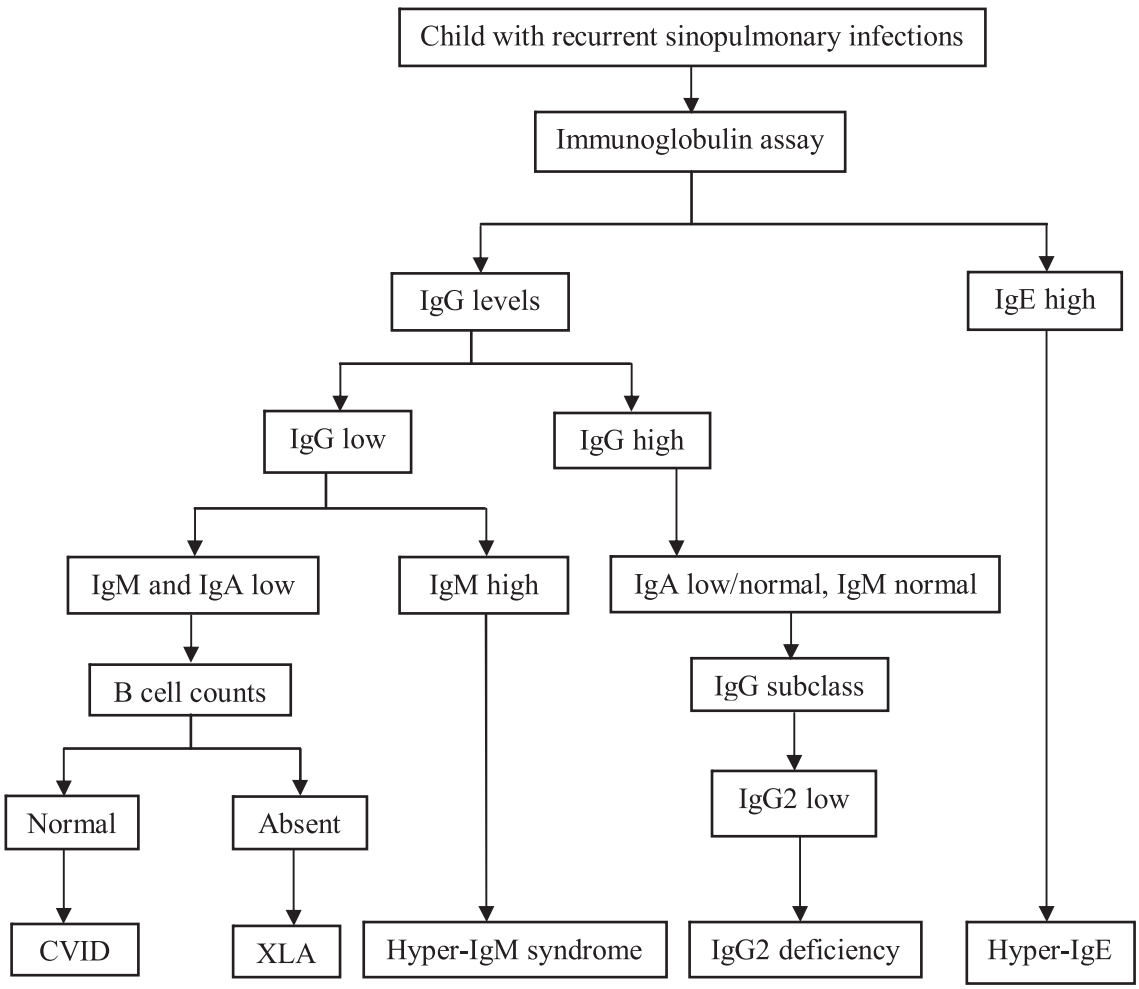


Fig.3. Utility of immunoglobulin assay in the diagnosis of PIDs

Humoral immune defect suspected and immunoglobulin profile was obtained:

IgG: 1650 mg/dL (608 - 1572)

IgA: 10 mg/dL (33 - 236)

IgM: 110 mg/dL (52 - 242)

IgA was reduced and IgM was high.

So, the diagnosis considered was IgA deficiency. However, IgA deficiency is known to present with a milder phenotype (repeated rhinitis and sinusitis). This child had recurrent pneumonia and hence further evaluation was considered. IgG subclass estimation was ordered.

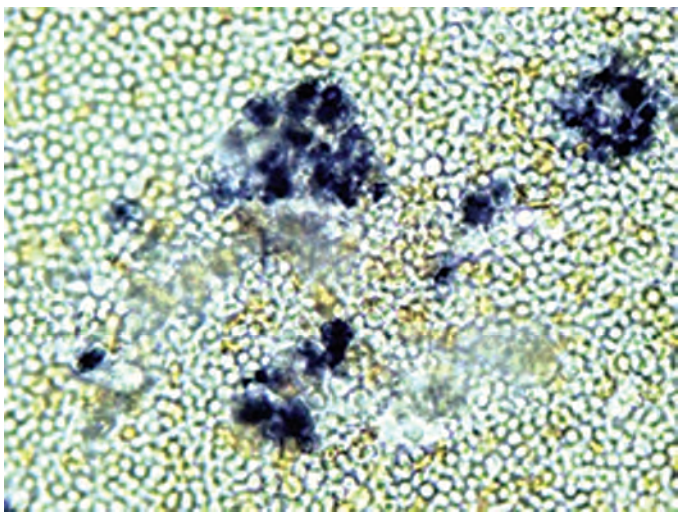


Fig.4. Bluish-black pigment (formazan granules) formation in slide indicates normal oxidative burst. This pigment formation will not be seen in a patient with CGD.

IgG1: 1425 mg/dL (423 - 939), IgG2: 45 mg/dL (70 - 426), IgG3: 150 mg/dL (27 - 207) and IgG4: 27 mg/dL (8 - 32).

Diagnosis: IgG2 subclass deficiency with IgA deficiency.

Message: In the setting of serious infections and IgA deficiency, one must evaluate for IgG subclass deficiency.

NBT and DHR tests

Nitro blue tetrazolium (NBT) test is a simple slide test used for screening patients with chronic granulomatous disease (CGD). The defect in the oxidative burst in leukocytes of patients with CGD may be identified by their failure to reduce NBT during phagocytosis (Fig.4).[19] But due to observer bias involved in interpreting this test, it is now agreed that the Dihydro rhodamine 123 (DHR) is the method of choice. DHR test is extremely robust and easy to perform and is an inexpensive test. Phagocytes from a healthy individual are capable of reducing DHR to fluorescent compounds. The DHR assay measures change in fluorescence of DHR loaded granulocytes after phorbol-myristate acetate (PMA) stimulation (Fig.5). This test can detect CGD patients, carriers and can suggest the genotype of the CGD patients.[20]

Case 11

A 6-year-old girl born to consanguineously married couple presented with fever and painful swelling on the right side of neck and was diagnosed with suppurative

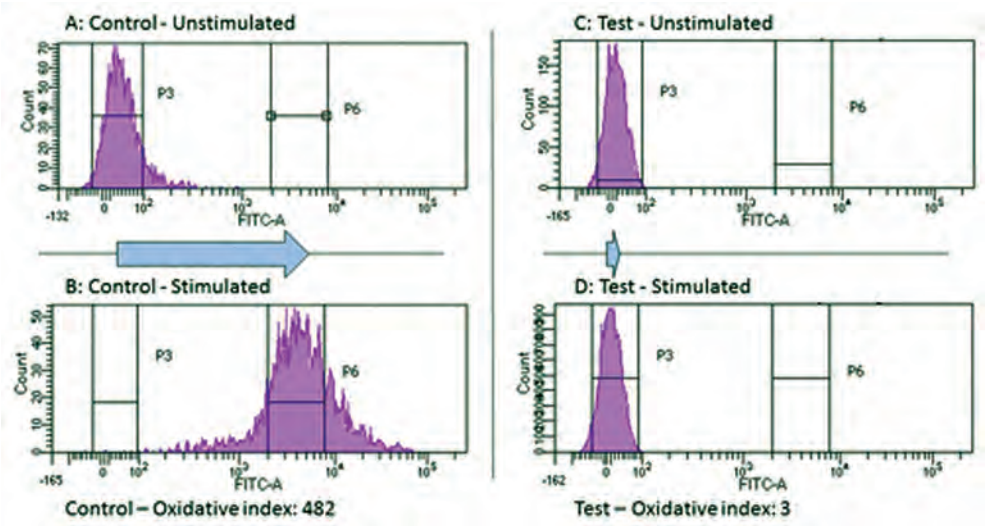


Fig.5. DHR assay in a patient with CGD in comparison to a healthy control.

cervical lymphadenitis. Siblings (9-year-old boy, 5-year-old girl) were healthy.

Pus was drained from the cervical node and culture grew Burkholderia cepacia. She was treated with antibiotics based on the sensitivity.

Past history: She had been treated for liver abscess at the age of 3 years.

Family history: Born to a consanguineously married couple.

In view of the unusual organism isolated during this admission and the significant past history, she was evaluated further.

Investigations were as follows:

HIV card test: negative

CBC Hb: 8.5g/dL, TC - 24,540/ mm^3 (N75 L15 M6 E4), PC - 6, 13,000/mm^3

ESR: 110 mm/hr

IgG: 2520 mg/dL (608 - 1572), IgA: 230 mg/dL (33 - 236), IgM: 324 mg/dL (43 - 207)

NBT and DHR tests were performed and showed reduced oxidative burst.

Genetic studies: Homozygous pathogenic mutation in neutrophil cytosolic factor 1(NCF1) gene.

Diagnosis: Chronic granulomatous disease (AR inheritance)

Message: In children presenting with suppurative infections - non resolving pneumonia/lung abscess/liver abscess/lymphadenitis, one must think of CGD.

Conclusion

PIDs, currently referred to as IEI, are not uncommon diseases. Patients with severe, unusual, persistent or recurrent infections must be investigated for an underlying PID. With the expanding spectrum, autoimmune and autoinflammatory manifestations of PIDs are increasingly being recognized. Contrary to common belief, PIDs are not restricted to the pediatric population and they may first manifest in adult life. A good clinical history, a detailed 3-generation pedigree analysis, followed by simple tests like CBC (taking special note of differentials, platelet counts and mean platelet volume) provide valuable clues to an underlying PID. Some of these tests, at times, assist in excluding a PID. For example, a normal platelet count excludes a possibility of WAS. Tests like serum immunoglobulins can assist in the diagnosis of humoral immune deficiencies. Immunoglobulin assay, NBT and DHR tests are now readily available at many centres. While genetic testing is often necessary to confirm the diagnosis, one must not delay initiation of therapy in these patients for want of genetic testing.

Points to Remember

- ***PIDs are currently referred to as IEIs.***
- ***One in 1000 individuals suffer from a PID, hence, these diseases are not rare.***
- ***The first step in the diagnosis of PIDs is history and clinical examination and to suspect them in clinical practice.***
- ***Careful analysis of routinely available tests like hemogram provide valuable clues to the underlying PID.***
- ***Neutropenia can be manifestation of a PID and noted in severe congenital neutropenia, cyclic neutropenia, hyper IgM syndrome and many other PIDs.***
- ***Marked neutrophilia must make one think of leukocyte adhesion deficiency.***
- ***Persistent thrombocytopenia in a male child warrants evaluation for possible Wiskott Aldrich Syndrome.***
- ***Serum immunoglobulin assay is a valuable tool in evaluation of patients with suspected PID, which has to be used more often in clinical practice.***
- ***NBT and DHR are simple screening tests for chronic granulomatous disease.***
- ***Once suspected, one must not delay initiation of therapy for want of genetic testing in these patients.***

References

1. McCusker C, Upton J, Warrington R. Primary immunodeficiency. Allergy, Asthma Clin Immunol 2018; 14(2):1-2.
2. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human inborn errors of immunity: 2019 update of the IUIS phenotypical classification. J Clin Immunol 2020; 11:1-6.
3. Bousfiha AA, Jeddane L, Ailal F, Al Herz W, Conley ME, Cunningham-Rundles C, et al. A phenotypic approach for IUIS PID classification and diagnosis: guidelines for clinicians at the bedside. J Clin Immunol 2013; 33(6): 1078-1087.
4. Modell V, Gee B, Lewis DB, Orange JS, Roifman CM, Routes JM, et al. Global study of primary immunodeficiency diseases (PI)-diagnosis, treatment, and economic impact: an updated report from the Jeffrey Modell Foundation. Immunol Res 2011; 51(1):61-70.

5. Parvez Y, Mathew AG. Hyperleukocytosis in newborn: a diagnosis of concern. Indian J Hematol Blood Transfus 2014; 30(1):131-132.
6. Michniacki TF, Walkovich KJ. Leukocytosis. In: Nelson Textbook of Pediatrics. Kliegman RM, St Geme JW, Blum NJ, Shah SS, Tisker RC, Wilson KM, Eds, 21st Edn, Vol.1. Philadelphia: Elsevier; 2020; pp1147-1149.
7. Kwan A, Church JA, Cowan MJ, Agarwal R, Kapoor N, Kohn DB, et al. Newborn screening for severe combined immunodeficiency and T-cell lymphopenia in California: results of the first 2 years. J Allergy Clin Immunol 2013; 132(1):140-150.
8. Celkan T, Koç B. Approach to the patient with neutropenia in childhood. Turk Pediatri Ars 2015; 50(3):136-144.
9. Welte K, Zeidler C, Dale DC. Severe congenital neutropenia. Semin Hematol 2006; 43(3):189-195.
10. Skokowa J, Germeshausen M, Zeidler C, Welte K. Severe congenital neutropenia: inheritance and pathophysiology. Curr Opin Hematol 2007; 14(1):21-28.
11. Berliner N, Horwitz M, Loughran TP Jr. Congenital and acquired neutropenia. Hematology Am Soc Hematol Educ Program 2004:63-79.
12. Dale DC, Hammond WP . Cyclic neutropenia: a clinical review. Blood Rev 1988; 2(3):178-185.
13. Rezaei N, Farhoudi A, Ramyar A, Pourpak Z, Aghamohammadi A, Moham- madpour B, et al. Congenital neutropenia and primary immunodeficiency disorders: A survey of 26 Iranian patients. J Pediatr Hematol Oncol 2005; 27:351-356.
14. Schmutz N, Henry E, Jopling J, Christensen RD. Expected ranges for blood neutrophil concentrations of neonates: the Manroe and Mouzinho charts revisited. J Perinatol 2008; 28:275-281.
15. Calhoun DA, Kirk JF, Christensen RD. Incidence, significance and kinetic mechanism responsible for leukemoid reactions in patients in the neonatal intensive care unit: a prospective evaluation. J Pediatr 1996; 129:403-409.
16. Nittala S, Subbarao GC, Maheshwari A. Evaluation of neutropenia and neutrophilia in preterm infants. J Matern Fetal Neonatal Med 2012; 25(Suppl 5):100-103.
17. Bosticardo M, Marangoni F, Aiuti A, Villa A, Grazia Roncarolo M. Recent advances in understanding the pathophysiology of Wiskott-Aldrich syndrome. Blood 2009; 113(25):6288-6295.
18. Zinkernagel RM. Maternal antibodies, childhood infections, and autoimmune diseases. N Engl J Med 2001; 345(18):1331-1335.
19. Battersby AC, Cale AM, Goldblatt D, Gennery AR. Clinical manifestations of disease in X-linked carriers of chronic granulomatous disease. J Clin Immunol 2013; 33(8):1276-1284.
20. Jirapongsananuruk O, Malech HL, Kuhns DB, Niemela JE, Brown MR, Anderson-Cohen M, et al. Diagnostic paradigm for evaluation of male patients with chronic granulomatous disease, based on the dihydrorhodamine 123 assay. J Allergy Clin Immunol 2003; 111(2):374-379.

CLIPPINGS

Efficacy of Prednisolone in recovery from Acute Bronchiolitis.

Children with acute bronchiolitis present with breathing difficulties, cough, poor feeding, and irritability. Most trials of bronchiolitis treatment suffer from 2 constraints: possible inclusion of patients with asthma and inconsistent outcome measures.

The aim of the study was to determine the efficacy of prednisolone in recovery from acute bronchiolitis who have a family history of atopy. This randomized double blind placebo controlled trial (RCT) was conducted with sixty (60) bronchiolitis patients having family history of atopy. The trial was so planned that neither the parents nor the investigator were aware of group allocation. The mean age of the patients of this series were 3.68 months (±1.29SD) and 3.52 month's (±1.1SD) in prednisolone and placebo group respectively. Use of accessory muscle score was assessed twice at 8 am and 8 pm each day for three days. First assessment on day 1 the score was similar in both the treatment groups (P>.05). More people in Prednisolone arm recovered within 3 days then the placebo group. The difference is statistically significant (P<.01).

Three-day oral prednisolone treatment was effective in accelerating clinical recovery.

Khondaker Zahirul Hasan, Md. Abid Hossain Mollah, Mohammad Monir Hossain, Muhammad Zahangir Alam, Shahidul Islam Bhuiyan ANM, Md. Faruk Ahmed. Efficacy of Prednisolone in Recovery from Acute Bronchiolitis: Study in a Tertiary Care Hospital, Dhaka, Bangladesh, Am J Pediatr Vol. 7, No.2, 2021, pp. 85-90. doi: 10.11648/j.ajp.20210702.19.

IMMUNOLOGY

INNATE IMMUNE DEFECTS

***Akshaya Chougule**
***Vaishnavi V. Iyengar**
****Vijaya Gowri**
*****Prasad Taur**
******Mukesh M. Desai**

Abstract: *The innate immune system is a phylogenetically ancient, germline-encoded system that enables eukaryotes to defend themselves against infections. It is "inborn" and does not require a developmental phase and forms the first line of defense against foreign material. It has an immediate or near immediate effect. Innate immune defects can be broadly classified as predisposition to invasive bacterial infections, predisposition to parasitic and fungal infections, Mendelian susceptibility to mycobacterial disease and predominant susceptibility to viral infections. Each defect has a narrow spectrum of infections and knowledge of the specific causative organism in each case helps in early diagnosis and therapeutic decision making.*

Keywords: *Inborn errors of immunity, Innate immunity, Toll-like receptor pathway, Mendelian susceptibility to mycobacterial disease.*

The innate immune system is a phylogenetically ancient, germline-encoded system that enables eukaryotes to defend themselves against infections.[1,2] Being termed as "inborn" it does not require a developmental phase and forms the first line of defense against foreign material. As no genetic recombination events are required to mediate its function, it has an immediate or near immediate effect.[1] Components of the innate immune system include anatomic barriers, soluble components such as proteins secreted onto mucosal surfaces or into the bloodstream and cellular components.[1]

* Clinical Fellow,
** Consultant,
*** Scientific Officer,
**** Head of Department Immunology,
Department of Pediatric Immunology,
Bai Jerbai Wadia Hospital for Children,
Parel, Mumbai,
Maharashtra, India.
email: mmdesai006@gmail.com

Innate immune response

The main strategy of the innate immune system is to recognize the patterns which are common to majority of the microorganisms.[1] These patterns include microbial associated molecular pattern (MAMP) and pathogen associated molecular patterns (PAMP) such as lipopolysaccharide (LPS) and double stranded RNA. In addition, danger associated molecular pattern (DAMPs) that are products of host DNA can also elicit an immune response. DAMPs are produced directly by host cells or produced in response to invading pathogens, cellular stress or malignancy. These patterns (PAMP, MAMP and DAMP) are recognized by pattern recognition receptors (PRR) such as Toll like Receptors (TLRs), C-type lectin receptor, nuclear binding oligomerization domain (NOD) leucine-rich-repeat containing receptor (NLRs) and retinoic acid-inducible gene-I protein (RIG-I) helicase receptor.[2] The TLR family consists of 11 members which are transmembrane proteins possessing leucine-rich repeats in their extracellular domain.[3] TLRs-1, 2, 4, 5, 6, 10 are located on the cell surface whereas TLRs-3,7,8,9 are intracellular and located within the endosomal compartment. TLR signaling occurs via two major pathways, the canonical pathway and the alternative pathway.[4]

The canonical pathway is the main signaling pathway for all TLRs except for TLR3 and the main adaptor protein involved in this pathway is MyD88 (Myeloid differentiation factor 88). The alternative pathway is the main signaling pathway for TLR3 and is independent of MyD88. TLR3 binds viral double-stranded RNA (dsRNA) and plays a vital role in anti-viral immunity.[4] (Fig.1) Mutations in the receptors or components of the innate immune signaling pathways result in distinct clinical phenotypes.

International Union of Immunological Societies (IUIS) classification

Defects in intrinsic and innate immunity fall under group VI of the IUIS classification and are broadly divided into two categories (Box 1) (Box 2).[5]

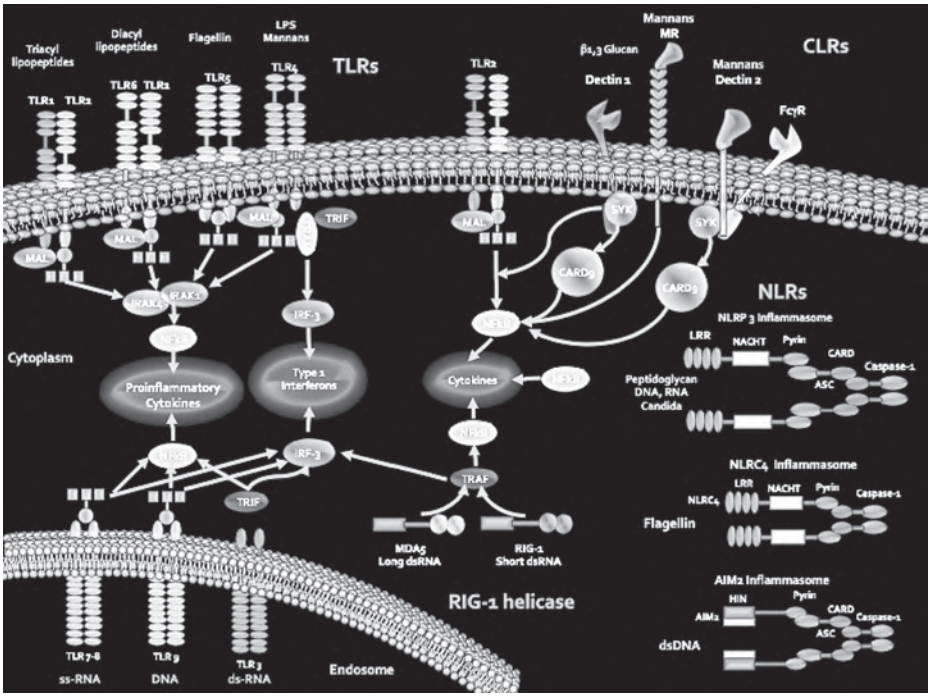


Fig.1. Pattern recognition receptors of the innate immune system and their signaling pathways.

(Source: Netea MG, van der Meer JW. Immunodeficiency and genetic defects of pattern-recognition receptors. N Engl J Med. 2011; 364(1):60-70).

Box 1. Categories of defects in intrinsic and innate immunity

a. Bacterial, parasitic and fungal infections which include

1. Predisposition to invasive bacterial infections (pyogenes)
2. Predisposition to parasitic and fungal infections

b. MSMD and viral infections which include

1. Mendelian susceptibility to mycobacterial disease (MSMD)
2. Predominant susceptibility to viral infections

Predisposition to invasive bacterial infections (pyogenes)

a) Interleukin-1 receptor (IL-1R)-associated kinase 4 (IRAK 4) and MYD88 deficiency.

- Mode of inheritance- These are inherited in an autosomal recessive (AR) manner and are phenocopies with respect to their immune phenotype.
- Clinical features - They have increased susceptibility to bacterial infections with normal immunity against viral, parasitic and fungal infections. The most common invasive infection is meningitis followed by sepsis, arthritis, deep seated abscess and osteomyelitis, and present early in life but become less frequent with age. Noninvasive infections continue into adulthood and include skin infections such as recurrent folliculitis, furuncles, cellulitis, sinus and upper respiratory tract infections including recurrent otitis media, gingivitis and pharyngitis. Characteristic clinical feature in these defects is that fever is subdued due to the lack of inflammatory markers.
- Most common causative organisms isolated- *Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa*.
- Laboratory evaluation- Normal CRP, lack of leukocytosis and normal levels of other inflammatory markers.
- Immunological workup - Peripheral blood leukocytes of these patients fail to produce IL-6 in response to IL-1β (a proinflammatory cytokine) and there is impaired response to pneumococcal vaccination.
- Medical management - Vaccination against *S. pneumoniae, H. influenzae and N. menigitidis*, prophylactic antibiotics and intravenous or subcutaneous immunoglobulin therapy to decrease the incidence of infections.[4]

Box 2. Defects in intrinsic and innate immunity

I. Bacterial and parasitic infections

1. Defects that predispose to invasive bacterial infections

a) Interleukin-1 receptor (IL-1R)-associated kinase 4 (IRAK 4)

b) MYD88 deficiency

c) Interleukin-1 receptor (IL-1R)-associated kinase 1 (IRAK1) deficiency

d) Toll-interleukin-1 receptor domain containing adaptor protein (TIRAP) deficiency

e) Isolated congenital asplenia

2. Predisposition to parasitic and fungal infections

a) Susceptibility to chronic mucocutaneous candidiasis (CMC)

b) caspase recruitment domain family member 9 (CARD9) deficiency - Susceptibility to invasive fungal infections

c) Other specific innate immune defects with CMC as one of the manifestations

d) Susceptibility to trypanosomiasis

II. Mendelian susceptibility to mycobacterial diseases (MSMD) and viral infections

1. Monogenic causes of MSMD

a) Defects affecting IFN-γ (Interferon - γ) production

Complete IL-12Rβ1 (Interleukin-12 receptor β1 subunit) deficiency, Complete IL-12p40 deficiency- AR (Autosomal Recessive), Complete IL-12Rβ2 deficiency-AR, Complete IL-23R deficiency - AD (Autosomal Dominant), Interferon regulatory factor 8(IRF8) deficiency - AR complete, IRF8 deficiency - AD, ISG15 deficiency - AR complete, Signal peptide peptidase-like 2 A (SPPL2a) deficiency, Janus Kinase 1(JAK1) loss of function, RORC (Retinoic Acid Receptor Related Orphan Receptor C) deficiency, T- bet deficiency (loss of function mutation in TBX21), AR complete IFN-γ deficiency, SerpinB1 deficiency).

b) Defects associated with poor response to IFN-γ (Interferon - γ) -

AR Complete IFN-γR1 deficiency, AR partial IFN-γR1, AD partial IFN-γR1 deficiency, AR complete IFN-γR2 deficiency, X-linked recessive B component of Cytochrome B(CYBB) ($gp91^{phox}$ or NOX2) deficiency, Autosomal recessive loss of function mutation in Zinc Finger NFX1 Type Containing 1(ZNFX1).

2. Predominant susceptibility to viral infection

a) The three single gene disorders underlying susceptibly to broad spectrum of infections - Signal transducer and activator of transcription-1 (STAT-1) loss of function mutations, TYK (Tyrosine Kinase 2) loss of function mutations, mutations in NEMO (NFκB essential modulator).

b) Single gene disorders with susceptibility to severe viral infections alone- IRF 7 loss of function mutations, Interferon- α Receptor- 1(IFNAR1) deficiency, IFNAR2 deficiency, STAT-2 deficiency, CD16 deficiency, melanoma differentiation-associated gene (MDA5) deficiency, RNA polymerase III deficiency, Interleukin-18 binding protein deficiency.

c) Susceptibility to Herpes Simplex Encephalitis (HSE) - Unc-93 Homology B1(UNC93B1) defect, Tumor necrosis factor receptor associated factor, TIR-domain-containing adaptor inducing interferon-β (TRIF) defect, IRF3 defect, TANK-binding kinase-1(TBK1S) defect, AD/AR mutations in TLR3, mutations in DBR1.

d) Predisposition to human papilloma virus (beta papillomavirus)- EVER1(epidermodysplasia verruciformis 1) deficiency, EVER2 deficiency, calcium and integrin binding protein- 1(CIB1) deficiency, WHIM (warts, hypogammaglobulinemia, infections and myelokathexis) syndrome.

b) Interleukin-1 receptor (IL-1R)-associated kinase 1 (IRAK1) deficiency.

- Mode of inheritance - Complete XR (X-linked Recessive) IRAK1 deficiency.
- Clinical features- The described case was a male infant with large Xq28 deletion. Unlike the invasive infections seen in IRAK4 and MyD88 deficiencies, this boy had infections such as conjunctivitis, urinary tract infections and rhinovirus infections which could be managed with standard treatment. However, this boy also had Rett syndrome and died at an early age.[6]

c) Toll-interleukin-1 receptor domain containing adaptor protein (TIRAP) deficiency.

- Mode of inheritance- Complete AR TIRAP deficiency is caused by homozygous mutations in the gene encoding TIRAP
- Defect- Mutations impair the binding of TIRAP to the receptor and MyD88. TIRAP is the adaptor protein required for signaling following activation of TLR 1/2, TLR 2/6, TLR4 and TLR10.
- Clinical features - Penetrance is incomplete, few cases may be asymptomatic. The reported case presented with pneumonia and sepsis with Panton-Valentine leucocidin producing *Staphylococcus aureus*.
- Laboratory evaluation - Little or no production of cytokines by the fibroblasts/ monocytes/ granulocytes following TLR2/ TLR4 ligation. There is also impaired immune response to lipoteichoic acid which is recognized specifically by TLR2 and TLR6. Patients also have deficiency of unswitched memory B cells which protect against encapsulated organisms such as *S. pneumoniae*.[6]

d) Isolated congenital asplenia

- Clinical features: These children are born with isolated absence of spleen with no other developmental anomaly.
- Causative organisms implicated: They are prone to invasive bacterial infections, especially with capsulated organisms such as *Streptococcus pneumoniae, N. meningitidis, H. influenzae and E. coli*.
- Inheritance: The genetic causes identified are autosomal dominant (AD) mutations in *NKX2-5* and *RPSA* genes.
- Management: Antibiotic prophylaxis against capsulated organisms such as long term oral penicillin prophylaxis. The child has to be immunized against *S. pneumoniae, H. influenzae, N. meningitidis* and conjugated vaccines are preferred.[7]

Predisposition to parasitic and fungal infections

a) Susceptibility to chronic mucocutaneous candidiasis (CMC)

- Definition: Chronic mucocutaneous candidiasis (CMC) is the recurrent or persistent infection of nails, skin, oral, oesophageal and genital mucosae caused by Candida species, most often *C. albicans*.
- Defects: Mucosal immunity depends on T-Helper 17(Th17) cells, γδ Tcells and Innate lymphoid cells (ILC3) expressing RORγT and the production of IL17. Deficiencies in IL17A, IL17F, IL17RA IL17RC (Interleukin 17 Receptor) and activator 1 of nuclear factor kappa - beta (ACT1) have been associated with CMC.[8]

b) Susceptibility to invasive fungal infections

- Pathway and associated defects - Protection against invasive fungal infections requires their recognition by host tissue pattern recognition receptors (Dectin-1, a C-type lectin). This allows the host cells to trigger downstream pathways that allow pathogen killing, leading to production of various cytokines that promote fungal killing by phagocytes especially neutrophils. Invasive fungal infections therefore occur with innate immune defects with CARD9 deficiency which is an important component of the Dectin pathway (Fig.1).[9]
- Phagocyte defects like neutropenia, chronic granulomatous disease (CGD) and leukocyte adhesion deficiencies (LAD) predispose to systemic infections with aspergillus (and other moulds) and candida.[9]

c) Other specific innate immune defects with CMC as one of the manifestations

- Autosomal dominant signal transducer and activator of transcription 1 (STAT1) gain-of-function (GOF) mutation
- Clinical features - It is associated with CMC, invasive fungal infections, sinopulmonary and cutaneous bacterial infections, viral infections (herpesviridae, molluscum contagiosum and warts). Many autoimmune manifestations in the form of autoimmune cytopenias, autoimmune endocrinopathies are also seen.[10]

d) IL-12 receptor β1 (IL-12Rβ1) deficiency, IL-12p40 deficiency, RORγT deficiency have been discussed in the section on MSMD.

Management of inborn errors of immunity associated with increased susceptibility to fungal infections -

- All cases need to be confirmed with culture.
- Azole therapy for ongoing infections and long term prophylaxis.
- Systemic fungal infections should be treated based on culture sensitivity and tissue penetration of drugs.
- Hematopoietic stem cell transplantation (HSCT) in those with monogenic disorders of immunity.[8,9]

D) Susceptibility to trypanosomiasis

- Mode of inheritance and genetic cause - Autosomal recessive apolipoprotein L1 (APOL1) deficiency has been reported in a male from India with a compound heterozygous mutation in *APOL1* gene.
- Causative organisms implicated - Opportunistic infection with *Trypanosoma evansi*, a non-pathogenic species, has been documented in the patient which was completely cured by Suramin therapy. APOL1 is required for the killing of non-pathogenic Trypanosoma species such as *T. evansi and T. brucei* but pathogenic Trypanosoma species are resistant to it. Thus, APOL1 deficiency is a specific defect in the innate immunity against non-pathogenic Trypanosoma species.[11]

Mendelian susceptibility to mycobacterial diseases (MSMD)

- Clinical features - These patients are otherwise healthy and are prone to infections even with attenuated *Mycobacterium bovis* - Bacillus Calmette-Guérin (BCG) vaccine strains. These patients usually present in childhood and rarely in adolescence and adulthood. The clinical spectrum ranges from localised to disseminated infections with one or more of the below mentioned pathogens that may or may not recur in the future.[12]
- Most common causative organisms implicated- Intracellular micro-organisms such as mycobacteria (M.Tb), non-tuberculous environmental mycobacteria (EM), bacteria such as non-typhi salmonella, salmonella, klebsiella, listeria, nocardia fungi such as candida, histoplasma, coccidioidomycosis and paracoccidioidomycosis, viruses such as cytomegalovirus (CMV), human herpes virus 8, parainfluenza virus type-3, respiratory syncytial and varicella zoster virus and parasites such as leishmania.[12,13]
- Pathway affected and implicated defects - Till date 21 genes and 37 clinical phenotypes have been identified. MSMD involves defects in the IL-12/23/ ISG15-IFN-γ axis.[13,14] (Fig.2) The IL-12/23/ ISG15-IFN-γ axis is required for the killing of

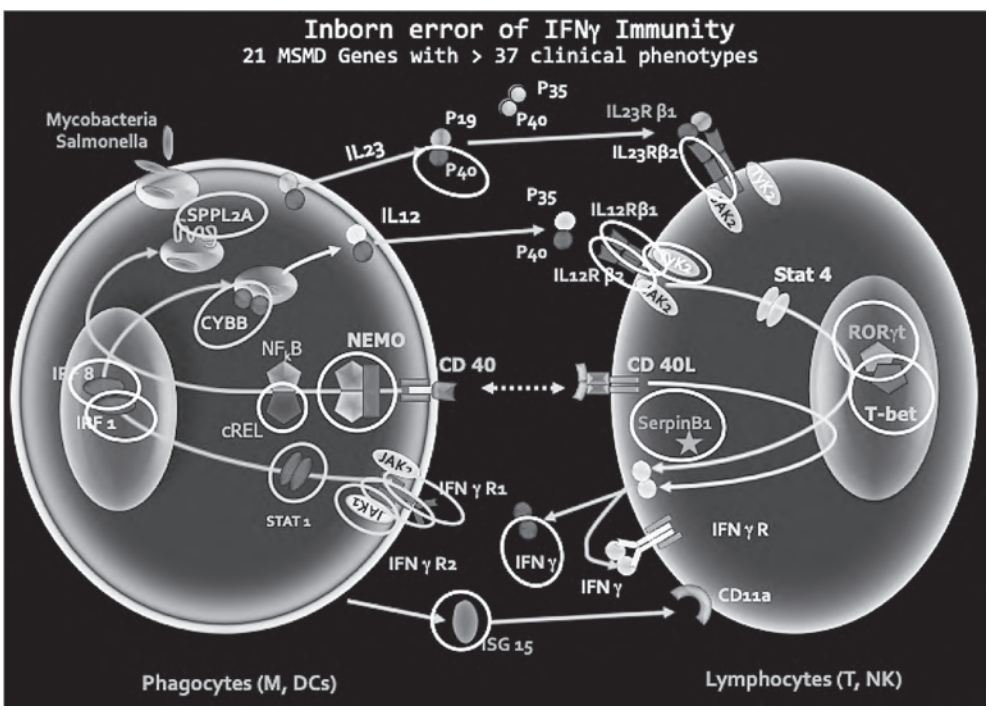


Fig.2. -IL-12/23/ISG15-IFN-γ axis. Encircled are the monogenic causes of MSMD.[12]

Table I. Description of the most common monogenic causes of MSMD

Defect	Infection profile	Immunological characteristics	Genetics	Treatment	Remarks
Defects affecting IFN-γ production					
Complete IL-12Rβ1 deficiency[12,13]	BCG-osis, EM, invasive Salmonellosis with leukocytoclastic vasculitis, *Klebsiella pneumoniae*, Pneumococcal disease and nocardiosis. Impaired IL-23-dependent IL-17 immunity predisposes these patients to mucocutaneous candidiasis	Absence of expression of functional receptor on activated T and NK cells Poor response to IL-12 and IL-23. Low serum levels of IFN-γ.	Autosomal recessive Low penetrance	Anti-tubercular therapy based on sensitivity pattern. BCG infection is Pyrazinamide resistant. IFN-γ therapy can be beneficial. Antibiotic prophylaxis is indicated in recurrent salmonellosis. HSCT can be offered to patients with a HLA-compatible donor in the family.	Most common genetic cause of MSMD in India and World. Clinical spectrum is variable ranging from severe disease with early death to asymptomatic presentation. Most common genetic cause for severe TB. BCG infection or vaccination protects against environmental mycobacteria (EM) infections and recurrent BCG infection is rare, suggesting that these patients have impaired immunity to primary mycobacterial infection but immunity against latent or secondary mycobacterial infection is intact.
AR Complete IL-12R β2 deficiency[12,13]	Disseminated BCG-osis and tuberculosis.	Absence of extracellular IL-12R β2 Impaired STAT4 phosphorylation	Homozygous mutations in the β2 subunit of IL-12 receptor Incomplete penetrance	Anti-tubercular therapy based on sensitivity pattern	Young age of onset
RORC deficiency[13]	Candidiasis and susceptibility to BCG infection and other mycobacteria, CNS tuberculoma,	Deficiency of RORγ or RORγT isoforms, both of which are important transcription factors required for	Germline loss of function mutations in *RORC* gene	Anti-tubercular therapy based on sensitivity pattern Antifungal therapy	

Defect		Infection profile	Immunological characteristics	Genetics	Treatment	Remarks
		oral thrush and onychomycosis	T lymphocyte development. Lack of these functional isoforms results in absence of IL-17A/F producing T cells which impairs immunity against Candida infection. Also there is poor production of IFN-γ by γδ T cells and CD4+CCR6 +CXCR3+αβ T cells which leads to defective immunity against mycobacterial infection			
Defects associated with poor response to IFN-γ						
IFN-γR1 defici-ency[12,13]	AR Complete IFN-γR1 deficiency	Disseminated, severe infections with BCG, M. tuberculosis and EM, Viruses such as CMV, HHV8, RSV, VZV, Bacteria such as *Listeria monocytogenes*, Salmonellosis is less common.	Lack of response to IFN-γ in vitro and plasma from these patients contains elevated levels of IFN-γ	Autosomal recessive	No response to exogenous IFN-γ therapy Only definitive therapy is HSCT, however the increased levels of IFN-γ predisposes to graft rejection.	Severe phenotype according to IUIS classification Early onset, before 3 years of age
	AR partial IFN-γR1	BCG-osis, EM such as *M. avium, M. abcessus, M. avium complex, M. szulgai*	Expression of IFN-γ receptors on the cell surfaces but impaired response to IFN-γ Abnormal STAT-1 phosphorylation.	Autosomal recessive	Require prolonged treatment with IV antibiotics Recombinant *IFN-γ*. HSCT is indicated only in patients with severe infections.	
	AD partial IFN-γR1 deficiency	BCG and EM, but have a less severe phenotype. Osteomyelitis is a common presentation in these patients	Accumulation of non-functional IFN-γR1 on the cell surface which fail to activate STAT-1 and JAK-1	Autosomal dominant	Anti-tubercular therapy based on sensitivity pattern	

Defect	Infection profile	Immunological characteristics	Genetics	Treatment	Remarks
AR complete IFN-γR2 deficiency[12,13]	BCG and EM Children present with ill-defined multibacillary granulomas. Salmonellosis CMV infections	Absence of IFN-γR2 Elevated levels of IFN-γ in serum and show poor response to exogenous IFN-γ	Autosomal recessive	HSCT is the only curative therapy	Phenotypically similar to Complete *IFN-γR1* deficiency with early onset Severe phenotype as per the IUIS classification Partial *IFN-γR2* deficiency is characterised by weak expression of *IFN-γR2* on the cell surfaces and have a less severe phenotype.

intra-macrophagic organisms and defects in this pathway increase susceptibility to the intracellular organisms mentioned above. Majority of the genetic mutations associated with MSMD either affect the production of IFN-γ such (IL12B, IL12RB1, IRF8, ISG15, NEMO) or response to it (IFNGR1, IFNGR2, STAT-1, IRF8, CYBB) and hence they are also known as inborn errors of IFN-γ immunity.[12-15] The most common monogenic causes of MSMD are mentioned in Table I.

Predominant susceptibility to viral infection

Interferons (IFN) are factors interfering with viral replication, apart from various other functions. They are of three types Human Type 1 IFNs, Human Type 2 IFN and Human Type 3 IFNs. Human Type 1 IFNs (IFN-α, β, ω, ε and κ) are responsible for antiviral response. The Type 2 IFN is IFN-γ which acts as a 'macrophage-activating factor' and defects in its pathway are associated with susceptibility to intra-cellular bacterial infections and mycobacterial disease as described above. Type 3 IFNs (IFN-λ 1, 2 and 3) have been shown to protect against some viral infections.[16]

Innate immune defects that lead to increased susceptibility to viral infections can be the broadly divided as.[16]

i. The three single gene disorders underlying susceptibly to broad spectrum of infections.

ii. Single gene disorders with susceptibility to severe viral infections alone.

iii. Susceptibility to Herpes simplex encephalitis (HSE).

iv. Predisposition to human papilloma virus.

i. The three single gene disorders underlying susceptibly to broad spectrum of infections

a. STAT1 (signal transducer and activator of transcription 1)

- STAT 1 is an important protein which is required for signal transduction and forms an integral part of most of the immune receptor signalling pathways.
- Autosomal recessive complete deficiency (loss of function mutation)

  Clinical features - Infantile onset disseminated BCG-osis, Mycobacterial infection, salmonella, HSV, EBV, RSV infection.
- Partial autosomal recessive loss of function mutations (Hypomorphic mutations)

Clinical features - Can come with similar disease spectrum as mentioned above but less in severity.

- Autosomal dominant loss of function mutations

Clinical features - Disseminated BCG, NTM. No viral infections.

Treatment of loss of function mutations in STAT-1

- Anti-Tubercular therapy based on sensitivity pattern.
- BCG - osis is inherently Pyrazinamide resistant.
- Acyclovir prophylaxis for recurrent HSV infections.
- Typhoid conjugate vaccine to prevent Salmonella infection, however non-typhoidal Salmonella infection can still occur.[16]

b. TYK 2 (Tyrosine Kinase 2) deficiency

- Genetic cause - autosomal recessive loss of function mutation
- Pathway involved- TYK2 is a member of Janus Kinase or JAK family. It is associated with various cytokine receptors which include IFN-α/β, IL6, IL12, IL23 and IL10 and is responsible for signal transduction involving JAK-STAT pathway. IL12/23 promote IFNγ production which is required for the elimination of intracellular organisms. Clinical features - Patients present with BCG-osis, recurrent and multiple viral infections, especially family Herpeviridae and intracellular organisms such as non-tubercular mycobacteria and Mycoplasma.[16]

c. NFκB essential modulator or NEMO defects

NFκB pathway is an important pathway involved in immune response and NEMO plays an important role in the activation of NFκB pathway. Mutations in NEMO are of 2 types -

1. X-linked recessive (hypomorphic) mutations -

   Affects males and is associated with Ectodermal dysplasia with Anhydrosis and Immune deficiency (EDA-ID). These patients are susceptible to bacterial, mycobacterial, viral and fungal infections.

2. X-linked dominant mutations -

   Affects females and patients present with incontinentia pigmenti or Behcet's disease.[16]

ii. Single gene disorders with susceptibility to severe viral infections alone

a. Autosomal recessive loss of function mutations in IRF 7 (Interferon Regulatory Factor- 7)

- IRF7 is the master regulator of type 1 IFN dependent immune responses.
- Clinical features - Isolated life-threatening, influenza infection in humans during primary infection.
- Management - Management of Influenza, other lifesaving measures.[17]

b. IFNAR1 (Interferon α Receptor 1) deficiency

- Associated with severe disease caused by Yellow Fever vaccine and Measles vaccine.
- 2 cases of IFNAR1 deficiency among persons with severe coronavirus disease 2019 (COVID-19).[18,19]

c. IFNAR2 (Interferon α Receptor 2) deficiency

- Reported case was a previously healthy child with fatal encephalitis following inoculation of the live-attenuated MMR vaccine. This child also had HHV6 infection.[18]

Other rare causes of increased susceptibility to viral infections include -

d. STAT2 Deficiency - Associated with disseminated disease with measles vaccine strain.[5]

e. CD 16 deficiency - NK (Natural Killer) cell defect associated with recurrent herpes viral disease.[5]

f. MDA5 deficiency (melanoma differentiation-associated gene) - Associated with susceptibility to Rhinovirus and other RNA viruses.[5]

g. RNA polymerase III deficiency- Associated with susceptible to severe VZV infection.[5]

h. IL 18 binding protein deficiency- Associated with fulminant viral hepatitis.[5]

iii. Susceptibility to Herpes simplex encephalitis (HSE)

- Inborn errors of TLR3 immunity can cause HSE. TLR3 recognizes dsRNA of viral origin and TLR3-IFN pathway is crucial for preventing development of HSE during primary infection with HSV type 1, between 3 months to 6 years of age.
- Defects downstream of TLR3- UNC93B1 defect (AR), TRAF3 defect (AD), TRIF defect (AD/AR), TBK1 defect (AD), IRF3 defect (AD).
- TLR3 mutations (AD/AR)- HSE and severe pulmonary influenza, VZV infection.
- DBR1 mutation - Debranching enzyme 1 (DRB1) encodes a RNA lariat debranching enzyme. Bi-allelic mutations are associated with brainstem encephalitis due to HSV-1, Influenza, norovirus and many other viruses.[16]

iv. Predisposition to human papilloma virus (beta papillomavirus)

a. EVER1 (epidermodysplasia verruciformis 1) deficiency, EVER2 (epidermodysplasia verruciformis 1) deficiency, CIB1 (calcium and integrin binding protein- 1) deficiency

- Infections and cancer of skin associated with EVER1 (epidermodysplasia verruciformis 1), EVER2 (epidermodysplasia verruciformis 1), CIB1 (calcium and integrin binding protein- 1) deficiency.

  EVER1 and EVER2 deficiency being the only non-hematopoietic immunodeficiency.[5]

b. WHIM syndrome

- The term WHIM is an acronym for the main signs of the syndrome: warts, hypogammaglobulinemia, infections and myelokathexis.
- Myelokathexis refers to impaired egress of mature neutrophils and other myeloid cells from the bone marrow, causing neutropenia.

  Signature pathogen in WHIM syndrome is human papillomavirus (HPV), which causes warts that cannot be controlled with standard medical treatment and may progress to cancer.[20]

Genetic susceptibility to SARS-CoV2

Recent studies conducted on the patients with severe COVID-19 showed that 3.5% of the patients had mutations in the genes required for host defence against viral infections such as influenza. These mutations included homozygous loss of function of IRF7 or IFNAR1, heterozygous mutations in *TLR3, TICAM1, TBK1* and *IRF3*. These genes are a part of the type-1 IFN pathway. Also, another study showed that 10% of the patients with severe COVID-19 had neutralizing auto-antibodies against type-1 IFN, associated with low level of type-1 IFN from the serum. Thus, inborn errors affecting type-1 IFN production or signalling pathway are associated with severe COVID-19.[19,21]

Conclusion

Monogenic defects of innate immunity involve complex protein defects which present with recurrent invasive and non-invasive infections in infancy and increased vulnerability to narrow spectrum of bacterial and viral pathogens. These defects have specific abnormalities seen on immunophenotyping however require molecular confirmation by next generation sequencing (NGS). Significantly improved outcome is seen with early diagnosis and institution of antibacterial, antifungal and antiviral prophylaxis and curative therapy in the form of HSCT when indicated. However, subtle and restricted immunological phenotype in each case is a clinical challenge.

Points to Remember

- ***Innate immune defects can be broadly classified as predisposition to invasive bacterial infections, predisposition to parasitic and fungal infections, Mendelian susceptibility to mycobacterial disease and predominant susceptibility to viral infections.***
- ***Interleukin 1 Receptor Associated Kinase 4 (IRAK4), Myeloid differentiation factor 88 (MyD88) and toll-interleukin 1 receptor (TIR) domain containing adaptor protein (TIRAP) deficiencies are associated with invasive bacterial infections.***
- ***Chronic mucocutaneous candidiasis is caused by defects in the IL-17 pathway, whereas defects in phagocytic defects and CARD9 deficiency cause invasive fungal infections.***
- ***Inborn errors in IFN-γ cause MSMD.***
- ***Errors in TLR3 signaling pathway and Type-1 Interferons lead to predisposition to viral infections and Type-1 Interferon pathway defects are also associated with severe COVID-19.***

References

1. Turvey SE, Broide DH. Innate immunity. J Allergy Clin Immunol 2010; 125(2):S24-32.
2. Hoffmann J, Akira S, Hoffmann J. Innate immunity editorial overview. Curr Opin Immunol 2013; 25(1):1-3.
3. Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunolog 2005; 17(1):1-14.
4. Sifers TM, Sampath V. Monogenic Defects of Toll-Like Receptor Signaling and Primary Immunodeficiency. In:Immunity and Inflammation in Health and Disease. Eds: Chatterjee S, Jungraithmayr W, Bagchi D. Academic Press Massachusetts 2018; 165-174.
5. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human inborn errors of immunity: 2019 update of the IUIS phenotypical classification. J Clin Immunol 2020; 11:1-16.
6. Boisson B. The genetic basis of pneumococcal and staphylococcal infections: inborn errors of human TLR and IL-1R immunity. Human Genetics 2020; 139(6):981-991.
7. Wong T, Yeung J, Hildebrand KJ, Junker AK, Turvey SE. Human primary immunodeficiencies causing defects in innate immunity. Curr Opin Allergy Clin Immunol 2013; 13(6):607-613.

8. Puel A, Cypowyj S, Maródi L, Abel L, Picard C, Casanova J-L. Inborn errors of human IL-17 immunity underlie chronic mucocutaneous candidiasis. Curr Opin Allergy Clin Immunol 2012; 12(6):616-630.
9. Glocker E-O, Hennigs A, Nabavi M, Schäffer AA, Woellner C, Salzer U, et al. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. N Eng J Med 2009; 361(18):1727-1735.
10. Zheng J, van de Veerdonk FL, Crossland KL, Smeekens SP, Chan CM, Al Shehri T, et al. Gainoffunction STAT1 mutations impair STAT3 activity in patients with chronic mucocutaneous candidiasis (CMC). Eur J Immunol 2015; 45(10):2834-2846.
11. Joshi PP, Shegokar VR, Powar RM, Herder SR, Salkar HR, et al. Human trypanosomiasis caused by Trypanosoma evansi in India: the first case report. Am J Trop Med Hyg 2005; 73(3):491-495.
12. Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinical features of inborn errors of IFN-γ immunity. Semin Immunol 2014; 26(6):454-470.
13. Taur PD, Gowri V, Pandrowala AA, Iyengar VV, Chougule A, Golwala Z, et al. Clinical and Molecular Findings in Mendelian Susceptibility to Mycobacterial Diseases: Experience From India. Front Immunol 2021; 12:426-438.
14. Casanova J-L, Abel L. Genetic dissection of immunity to mycobacteria: the human model. Annu Rev Immunol 2002; 20(1):581-620.
15. Bustamante J. Mendelian susceptibility to mycobacterial disease: recent discoveries. Human Genetics 2020; 139(6):993-1000.
16. Sancho-Shimizu V, de Diego RP, Jouanguy E, Zhang S-Y, Casanova J-L. Inborn errors of anti-viral interferon immunity in humans. Curr Opin Virol 2011; 1(6):487-496.
17. Ciancanelli MJ, Huang SXL, Luthra P, Garner H, Itan Y, Volpi S, et al. Life-threatening influenza and impaired interferon amplification in human IRF7 deficiency. Science 2015; 348(6233):448-453.
18. Duncan CJA, Mohamad SMB, Young DF, Skelton AJ, Leahy TR, Munday DC, et al. Human IFNAR2 deficiency: Lessons for antiviral immunity. Sci Transl Med 2015; 7(307):307ra154-162.
19. Zhang Q, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, Chen J, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science 2020; 370(6515):eabd4570.
20. Hernandez PA, Gorlin RJ, Lukens JN, Taniuchi S, Bohinjec J, Francois F, et al. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. Nat Genet 2003; 34(1):70-74.
21. Tangye SG, Al-Herz W, Bousfiha A, Cunningham-Rundles C, Franco JL, Holland SM, et al. The ever-increasing array of novel inborn errors of immunity: an interim update by the IUIS committee. J Clin Immunol 2021; 41(3):666-679.

CLIPPINGS

Multisystem Inflammatory Syndrome in Children - Initial Therapy and Outcomes.

The assessment of real-world effectiveness of immunomodulatory medications for multisystem inflammatory syndrome in children (MIS-C) may guide therapy. The authorsanalyzed surveillance data on inpatients younger than 21 years of age who had MIS-C and were admitted to 1 of 58 U.S. hospitals between March 15 and October 31, 2020. The effectiveness of initial immunomodulatory therapy (day 0, indicating the first day any such therapy for MIS-C was given) with intravenous immune globulin (IVIG) plus glucocorticoids, as compared with IVIG alone, was evaluated with propensity-score matching and inverse probability weighting, with adjustment for baseline MIS-C severity and demographic characteristics. The primary outcome was cardiovascular dysfunction (a composite of left ventricular dysfunction or shock resulting in the use of vasopressors) on or after day 2. Secondary outcomes included the components of the primary outcome, the receipt of adjunctive treatment (glucocorticoids in patients not already receiving glucocorticoids on day 0, a biologic, or a second dose of IVIG) on or after day 1, and persistent or recurrent fever on or after day 2.

Among children and adolescents with MIS-C, initial treatment with IVIG plus glucocorticoids was associated with a lower risk of new or persistent cardiovascular dysfunction than IVIG alone.

Son MBF, Murray N, Friedman K, Young CC, Newhams MM, Feldstein LR, et al. Multisystem Inflammatory Syndrome in Children - Initial Therapy and Outcomes. N Engl J Med. Downloaded from nejm.org on June 29, 2021.

IMMUNOLOGY

PRIMARY ANTIBODY DEFICIENCIES

***Sathishkumar Loganathan**
***Murugan Sudhakar**
****Pandiarajan Vignesh**
***** Surjit Singh**

Abstract: *Primary antibody deficiencies are a group of primary immunodeficiency disorders characterized by a marked reduction or absence of serum immunoglobulins due to intrinsic genetic defects in B-cells or impaired interaction between B-cells and T- cells. Clinical symptoms first manifest usually around 6-12 months of life when maternally acquired antibody levels are waning. The sine qua non of antibody deficiency syndromes is recurrent sino-pulmonary infections, especially with encapsulated organisms.Replacement with intravenous immunoglobulin is the mainstay of treatment in primary antibody deficiencies.*

Keywords: *Agammaglobulinemia, Primary immunodeficiency, Recurrent infections, Hypogammaglobulinemia.*

Primary immunodeficiency disorders (PIDs) or inborn errors of immunity (IEI) are inherited disorders that impair the immune response, leading to increased risk of infections, autoimmunity, immune dysregulation, inflammation or malignancy.The estimated prevalence of PID ranges between 1:8500 to 1:100000 for symptomatic patients.[1] In India approximately 1 million patients are likely to have PID. Primary antibody deficiencies (PAD) are a heterogenous group of common PIDs characterized by recurrent sino-pulmonary,and gastrointestinal infections (Table1) . Molecular defects intrinsic to B cell development and function or T-B interaction usually result in PAD. Hypogammaglobulinemia is the characterisitic feature of PAD, and it is secondary to variable defects in B-cell number, or function or due to loss of antibody function.

* Senior Resident

** Assistant Professor

*** Head - Department of Pediatrics, Professor In-Charge, Allergy Immunology Unit, Department of Pediatrics, Advanced Pediatrics Centre, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

email: vigimmc@gmail.com

B cell development

The common lymphoid progenitor cells (CLP) that are destined to be B cells, undergo the following stages of development in bone marrow: Pro B cells to Pre-B cell stage, and then immature B cells. In spleen, immature B cells undergo the following stages of development: transitional cells, mature B cells and immunoglobulin secreting plasmablasts upon recognition of antigen. Stimulated B cells undergo class-switch recombination in germinal centres for isotype switching of immunoglobulins. The most common PADs with defects in the development of B cells is X- Linked agammaglobulinemia (XLA) with defects in Bruton tyrosine kinase (BTK) protein required for differentiation of Pro-B cells to Pre-B cells. In common variable immunodeficiency (CVID), one or the other defects in B cell activation/ differentiation/ terminal differentiation of B cells to plasma cells and/or memory B-cells were reported. Class switch recombination is impaired in Hyper IgM syndrome resulting in failure of isotype switching from IgM to other immunoglobulins.

Hypogammaglobulinemia can be also be secondary to the following causes: protein losing enteropathy, nephrotic syndrome, intestinal lymphangiectasia, and severe burns. Common drugs that cause hypogammaglobulinemia include carbamazepine, phenytoin and corticosteroids. Various classes of PADs are described in Table I and a flow-diagram approach is described in Fig.1.

X -Linked agammaglobulinemia

XLA is a common PAD, initially described by Colonel Ogden Bruton in 1952. It is an X-linked inherited disease due to mutations in the *BTK* gene. BTK protein is expressed in B cell lineage and has a crucial role in early B cell development. As a result, there is failure of B cell development and affected patients have significantly reduced levels of (<1%) of lymphocytes.

Clinical manifestations

XLA is characterized by recurrent upper and lower respiratory tract infections (pneumonia, otitis media,

Table I. Categories of B cell immunodeficiencies

Spectrum	Immunoglobulin levels and B cells	Types or known defects
Agammaglobulinemia	Severe reduction in all isotypes of serum immunoglobulins with markedly reduced B cells	• X-linked agammaglobulinemia (XLA) • Autosomal agammaglobulinemia
Common variable Immunodeficiency	Reduction in IgG and either IgM/ IgA with normal or low number of B cells	• Transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) deficiency • B cell activating factor (BAFF) receptor deficiency • TNF-like weak inducer of apoptosis (TWEAK) deficiency • Inducible co-stimulator (ICOS) deficiency • CD 19 deficiency
Hyper IgM syndrome	Severe reduction in serum IgG and IgA with normal/ elevated IgM with normal number of B cells	• CD 40 Ligand deficiency • CD 40 deficiency • Activation-Induced Cytidine Deaminase (AID) deficiency • Uracil-N-glycosylase (UNG) deficiency
IgG subclass deficiency	Isotype or light chain deficiencies with generally normal numbers of B cells	IgG1, IgG2, IgG3, and IgG4 deficiency
Transient hypogammaglobulinemia	Reduced serum IgG, IgA and IgM for age	Transient hypo-gammaglobulinemia of Infancy
Specific antibody deficiency with normal immunoglobulins	Normal serum immunoglobulins; normal numbers of B cells; inability to produce antibodies to specific antigens	Specific antibody deficiency

sinusitis, bronchitis, and gastroenteritis).[2-4] Absent or atrophied rudimentary tonsils and non-palpable lymph nodes are characteristic clinical pointers in clinching the diagnosis. The European Society of Immunodeficiencies (ESID) diagnostic criteria of XLA is summarized in Table II.

Absent lymph nodes and tonsils are due to failure in generating plasma cells and B cells due to mutation in *BTK*. Encapsulated organisms like *Streptococcus pneumoniae, Streptococcus pyogenes, Hemophilus influenzae* and *Pseudomonas sp.*, are most commonly reported in XLA.[5] Other reported organisms include Giardia lamblia (chronic diarrhea), enterovirus (meningoencephalitis) and Mycoplasma sp., (respiratory, urogenital and joint infection) (Table III). Clinical features of XLA usually manifest at around 6-12 months of age, as transplacentally acquired maternal immunoglobulins are removed from the circulation at this age. Most patients present with history of recurrent infections and > 50% of children have had serious infections by 2 years of life.[3]

Laboratory diagnosis

Pan-hypogammaglobulinemia (IgG, IgA and IgM) with marked reduction of peripheral (B cells less than 2% of lymphocytes) is the hallmark of XLA. Post-vaccination antibody titres against polysaccharide/ protein antigens are either undetectable or markedly reduced. Both the function, and number of T cells are unaffected in XLA.[3,6] Flow cytometric analysis of intra-cellular expression of BTK protein in monocytes is a simple and rapid test to support the diagnosis of XLA. However, a normal level of Btk protein expression cannot rule out XLA, as functional defects in Btk protein are seen in around 20% of patients with XLA. Hence, genetic analysis is needed to confirm the diagnosis of XLA and it is especially crucial in patients with normal Btk expression but strong clinical suspicion. Neutropenia has been reported in 10-25% patients of XLA. However, the etiology for neutropenia in XLA remains unclear.

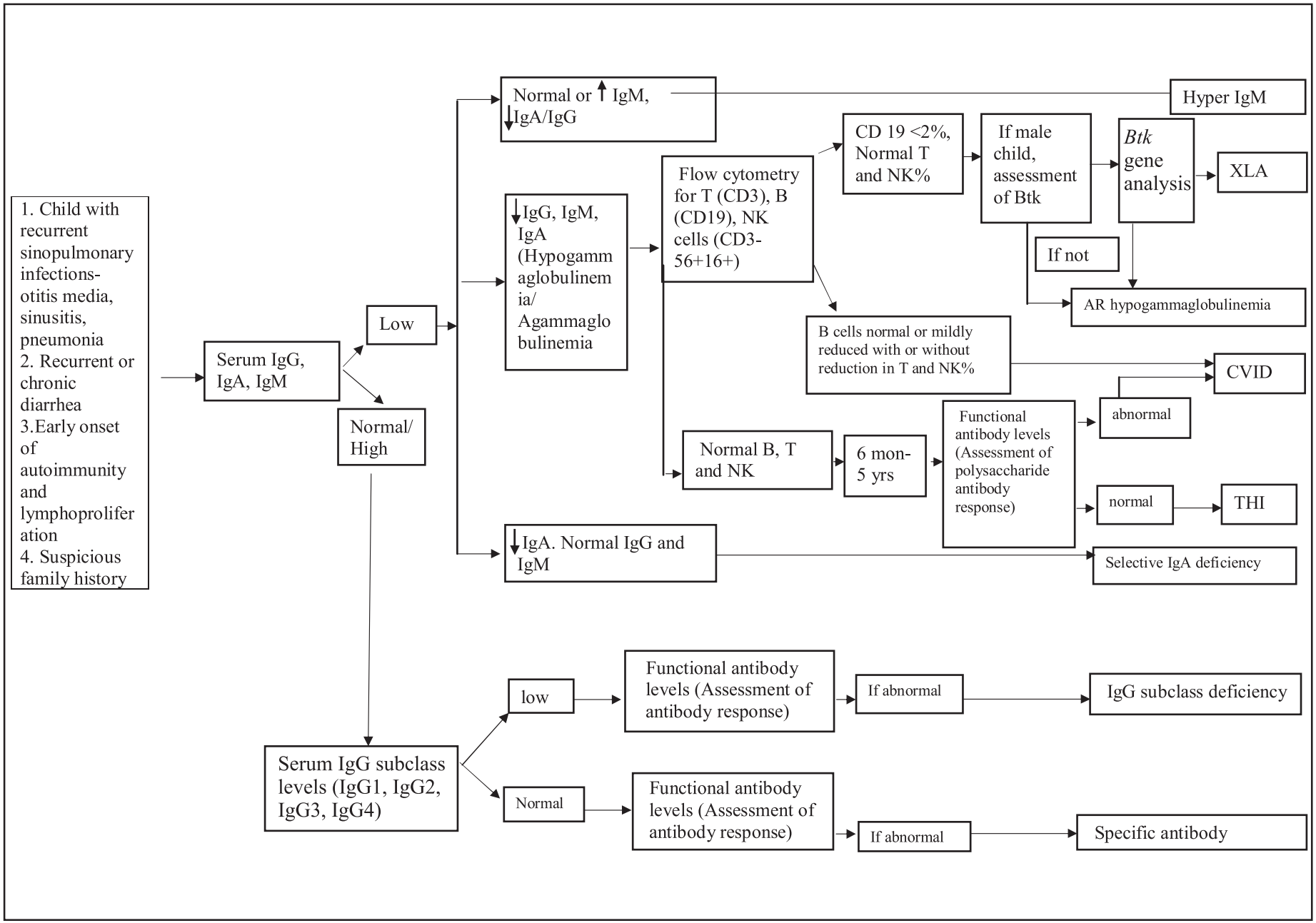


Fig.1. Approach to Primary B cell immune deficiency disorder

Table II. European society for immunodeficiencies (ESID) definition for XLA

Definitive
Male patient with less than 2% of CD19+ B cells and at least one of the following:

1) Mutation in *BTK*
2) Absent Btk mRNA on northern blot analysis of neutrophils or monocytes
3) Absent Btk protein in monocytes or platelets
4) Maternal cousins, uncles or nephews with less than 2% of CD19+ B cells

Probable
Male patient with CD19+ B cells of less than 2% and fulfils all the following:

1) Onset of recurrent bacterial infections in the first 5 years of life
2) Serum IgG, IgM and IgA > 2SD below normal for age
3) Absent isohemagglutinins and /or poor response to vaccines
4) Other causes of hypogammaglobulinemia have been excluded

Possible
Male patient with less than 2% CD19+ B cells in whom other causes of hypogammaglobulinemia have been excluded and fulfils at least one of the following:

1) Onset of recurrent bacterial infections in the first 5 years of life
2) Serum IgG, IgM and IgA more than 2 SD below normal for age
3) Absent isohemagglutinins

Table III. Clinical features of XLA

	Clinical manifestations	Organisms
Infections	Sino-pulmonary infections	*Streptococcus pneumoniae, Hemophilus influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa, Staphylococcus aureus, Neisseria meningitidis*
	Otitis media	
	Osteomyelitis	
	Meningitis	
	Arthritis	*Mycoplasma pneumoniae*
	Chronic diarrhoea	*Giardia lamblia, Campylobacter jejuni*
	Meningoencephalitis	Enteroviruses
Autoimmunity	Inflammatory bowel disease; Arthritides	
Malignancy	Non-Hodgkin lymphoma; Colorectal cancer	
Others	Glomerulonephritis; Growth hormone deficiency	

Management

The following factors need to be focused on while managing a patient of XLA.

A. Treatment of infections

B. Replacement therapy with immunoglobulin G (intravenous/ subcutaneous)

C. Prophylactic antibiotic therapy

Replacement therapy with immunoglobulin G

Immunoglobulin G (IgG) replacement therapy is the mainstay of management in XLA. Intravenous formulations (IVIg) are the most widely used form of replacement therapy till date and recently, subcutaneous immunoglobulin preparation (SCIG) was launched in India. The usual replacement dose of IVIg is 400 mg/kg (range: 300 to 600 mg/kg) by infusion once in every 3 to 4 weeks.

Dose of IVIg is tailored to maintain the biological trough level range of the patient.[7] Goal of IVIg is to prevent infections, not to achieve IgG trough level.

Breakthrough infections in XLA are secondary to inadequate dose of IVIg replacement or non-compliance with recommended therapy. Judicious and aggressive use of antimicrobials, efforts to isolate organism and antibiotic sensitivity pattern is important in managing patients with XLA who had breakthrough infections. Prolonged course of antimicrobials may be required in patients with sinusitis and ongoing pneumonia. Oral cotrimoxazole (5 mg/kg of trimethoprim/day) prophylaxis is often used concomitantly with immunoglobulin replacement therapy.

Live vaccines are contraindicated in patients with XLA. For patients who are on regular IVIg replacement, annual influenza vaccine is recommended. Inactivated vaccines can be given even in patients who are not on regular IVIg.

Common variable immunodeficiency (CVID)

CVID is a heterogenous group of PAD, characterized by hypogammaglobulinemia (low levels of IgG, and either low IgA or IgM) and absent to poor antibody responses to vaccination or low isohemagglutinin titres.[8,9] Clinical manifestations include both infectious and non-infectious conditions. Symptoms usually start after first decade of life and diagnosis may often get delayed due to the protean manifestations of CVID.[9] The usual clinical features include recurrent bacterial infections, varied autoimmune features, lymphoproliferation, malignancy, enteropathy, and allergic disease (Table IV).[10,11] Due to the myriad clinical manifestations, it is not surprising that many children with CVID land up with specialties other than immunology before a diagnosis can be established.[12] Consanguinity and family history of autoimmune disease or recurrent infections suggestive of PADs is common in patients with CVID.[9,13] Several monogenic causes have now been identified in patients with CVID.[14] Such patients may have unique clinical phenotypes, and are summarized in Table V.[15] Identifying monogenic causes is important because specific immunomodulatory therapies are available for management of autoimmunity and lymphoproliferation.

Clinical manifestations

In contrast to XLA, patients with CVID have normal sized or enlarged tonsils and 25% of patients have generalized lymphadenopathy and splenomegaly. Diagnosis of CVID in early age (less than 4 years) is problematic in view of immunological immaturity. Diagnostic criteria are summarized in Table VI. Differential diagnosis of CVID in early childhood includes

Table IV. Clinical manifestations of CVID

	Spectrum	Manifestations
Infections	Otitis media and recurrent pneumonia	*Streptococcus species, Haemophilus species, Moraxella catarrhalis, Neisseria meningitides* and *Staphylococcus*
	Acute and chronic diarrhea	*Giardia lamblia, Campylobacter jejuni, Salmonella, Norvo virus, Cryptosporidium parvum Cytomegalovirus* and *Helicobacter pylori*
	Arthritis	*Mycoplasma*
Autoimmune manifestations	Thrombocytopenia, Autoimmune hemolytic anemia / Neutropenia	
Polyclonal lymphoproliferation	Granuloma, Lymphocytic interstitial pneumonia, Persistent unexplained lymphadenopathy	
Malignancy	Extra nodal non-Hodgkin lymphoma, Chronic atrophic gastritis and metaplasia, Gastric cancer	
Allergic disease	Asthma, Allergic rhinitis, Atopic dermatitis, Allergic eczema, Food allergy, Urticaria, Allergic conjunctivitis and drug allergy	
Others (CNS and Liver)	Autoimmune and inflammatory myelitis, Primary biliary cholangitis, Granulomatous liver disease and Non-cirrhotic portal hypertension (including nodular regenerative hyperplasia)	

Table V. Monogenic causes of CVID

Gene	Inheritance	Clinical manifestations
ICOS	AR	Autoimmune neutropenia, splenomegaly, lymphadenopathy, enteropathy and interstitial lung disease
CTLA4	AD	Respiratory infections, diarrhea and lymphoid organ infiltration
LRBA	AR	Autoimmunity, splenomegaly, lymphadenopathy, enteropathy and interstitial lung disease
PIK3CD	AD	Recurrent respiratory infections, bronchiectasis, severe herpesvirus infections and lymphoma
TACI	AR/AD	Autoimmunity (ITP, AIHA), splenomegaly, lymphadenopathy and granulomatous disease
BAFF-R	AR	Infections only
TWEAK	AD	Recurrent respiratory infections, pneumococcal meningitis and warts
NFKB2	AD	Recurrent respiratory infections, meningococcal meningitis, adrenal insufficiency and Immune thrombocytopenia purpura

Abbreviations:*ICOS- Inducible co-stimulator, CTLA4- cytotoxic T lymphocyte antigen; LRBA-Lipopolysaccharide responsive beige-like anchor protein;PIK3CD-Phosphatidylinositol-4,5-Biphosphate 3-Kinase Catalytic Subunit Delta;TACI- TACI-Transmembrane activator and calcium-modulator and cyclophilin ligand interactor;BAFF-R–B- cell activating factor- Receptor;TWEAK- TNF- like weak inducer of apoptosis;NFKB2- Nuclear Factor Kappa B subunit 2.*

Table VI. ESID Criteria for CVID

Probable
Male or female patient who has a marked decrease of IgG (at least 2 SD below the mean for age) and a marked decrease in at least one of the isotypes IgM or IgA, and fulfils all of the following criteria: 1) Onset of immunodeficiency at greater than 2 years of age 2) Absent isohemagglutinins and/or poor response to vaccines 3) Defined causes of hypogammaglobulinemia have been excluded
Possible
Male or female patient who has a marked decrease (at least 2 SD below the mean for age) in one of the major isotypes (IgM, IgG and IgA) and fulfils all of the following criteria: 1) Onset of immunodeficiency at greater than 2 years of age 2) Absent isohemagglutinins and/or poor response to vaccines 3) Defined causes of hypogammaglobulinemia have been excluded

transient hypogammaglobulinemia of infancy (THI) - a benign and self limited condition in most cases.

Laboratory diagnosis

Hypogammaglobulinemia – markedly decreased IgG, with either low IgA / IgM levels and poor antibody response to vaccination are the classical features noted in patients with CVID. Flow cytometry analysis reveals normal to low B cell and normal T cell numbers. Reduced numbers of class-switched memory B cells ($CD19^+$ $CD27^+$ IgM^- IgD^-) (< 1-2% of $CD\ 19^+$ B cells) is noted in many patients with CVID. Molecular assays are usually needed to identify monogenic forms of CVID. Patients with CVID may produce organ specific autoantibodies (eg., including Coombs tests, antithyroid peroxidase and thyroglobulin antibodies) despite overall antibody deficiency.

Management

Management incudes IVIg replacement therapy and prophylactic antimicrobials (cotrimoxazole). Some patients with CVID may also require immunosuppressive agents (e.g. steroids/other immunosuppressive agents) or biologics

(rituximab, infliximab and abatacept) for auto-immune manifestations. Corticosteroids are useful in patients with CVID who have granulomatous complications. Treatment of monogenic forms of CVID includes cytotoxic T lymphocyte antigen-4 (CTLA-4) agonists abatacept and belatacept, mTOR inhibitors, TNF inhibitors.

Monitoring for subclinical pulmonary complications is important since patients can develop chronic pulmonary disease.

Selective IgA deficiency (SIgAD)

SIgAD is the most common PID, but it is usually asymptomatic. ESID defining criteria for SIgAD include: Level of serum IgA < 7 mg/dL in patients aged > 4 years with normal range of serum IgG and IgM; normal vaccine responses and with the exclusion of secondary causes of hypogammaglobulinemia and T-cell defects. Evaluation for SIgAD should be contemplated in patients with anaphylactic reaction to blood products and in patients with family history of PAD. Severe infections with SIgAD are usually associated with IgG subclass deficiencies (IgG2 and IgG4).

IV Ig replacement therapy could be considered inpatients with concomitant specific antibody deficiency and patients with poor response to antibiotic prophylaxis. IVIg with low IgA content is preferred in these patients.[16]

Hyper IgM syndrome

Hyper IgM syndrome (HIGM) is an uncommon PID categorized by normal or increased levels of serum IgM and low or absent levels of IgG, IgA and IgE. Various X linked, autosomal dominant/recessive inheritance forms are reported in HIGM. Signature organisms of HIGM include Pneumocystis jiroveci and cryptosporidium parvum.This syndrome is caused by a defect in class switch recombination. In HIGM there is defective interaction of CD40L with CD40 between CD4 T cells and B cells.

Clinical manifestations are heterogenous (Table VII), and usually start in infancy. Severe infections are observed in patients with CD 40/CD40L deficiencies, especially with opportunistic organisms like *Pneumocystis jiroveci*, *Cryptosporidium parvum*, *Bartonella*, Herpes family viruses and Enteroviral meningoencephalitis. Recurrent respiratory tract infections are common to both X-lined and other forms of HIGM- pneumonia and it is one of the commonest manifestation and complication in these patients. Gastrointestinal manifestations are the second most common feature in HIGM, especially chronic diarrhea with organisms like Giardia lambia, C.parvum, Salmonella spp. and Entamoeba histolytica are frequently reported. Other manifestations like proctitis, recurrent oral, gingivitis and perianal ulcers are secondary to neutropenia in CD40/CD40L deficiency. The spectrum of autoimmune

Table VII. Clinical manifestations of HIGM

Type	Inheritance	Clinical manifestations	Gene/protein	Immunology Laboratory findings
HIGM 1	X-linked	Chronic diarrhea (Pneumocystis jirovecii, Cryptosporidium sp.) Sclerosing cholangitis	*CD40LG*; TNFSF5CD154; CD40L	Neutropenia Increased IgM Decreased IgG Decreased IgA Absent/poor vaccine responses
HIGM2 & HIGM 4	AR/AD	Recurrent URTI & LRTI, lymphadenopathy, splenomegaly autoimmunity: hemolytic anemia, Thrombocytopenia	*AICDA*; AID	Decreased IgG and IgA Increased IgM Absent/poor vaccine responses
HIGM 3	AR	Recurrent bacterial infections (P.jirovecii and Cryptosporidium), Sclerosing cholangitis	CD40;CD40 Increased IgM	Neutropenia Decreased IgG and IgA
HIGM 5	AR	Bacterial infections and lymphadenopathy	UNG	Increased IgM Decreased IgG and IgA Absent/poor vaccine responses

manifestations include Coombs positive hemolytic anemia, autoimmune hepatitis, inflammatory bowel disease and sero-negative arthritis.

Lymphoproliferation and malignancies are reported in patients with activation induced cytidine deaminase (AID) and uracil-N-glycosylase (UNG) defects due to over-activated germinal centers. These patients are prone to develop lymphoma, adenocarcinoma of GI tract, liver malignancies (bile duct carcinoma, hepatocellular carcinoma, and biliary tree tumors) are commonly reported in X-linked HIGM.

Diagnosis

Decreased IgG and IgA with normal or elevated IgM is suggestive of HIGM. Presence of neutropenia points towards X-linked HIGM. Decreased expression of CD40L (CD 154) on activated $CD4^+$ T cells by flow cytometry is seen in X-linked HIGM. Molecular analysis is needed for genetic confirmation.

Management

Treatment involves IVIg replacement therapy, antimicrobial therapy for infections and granulocyte-colony stimulating factor (G-CSF) for neutropenia. Immunosuppressive therapy is often required for patients with auto-immune cytopenias. Trimethoprim-sulfamethoxazole antimicrobial prophylaxis is recommended for decreasing incidence of intercurrent infections.

Transient hypogammaglobulinemia of infancy (THI)

THI is a relatively uncommon disorder. It is an exaggeration of the physiological nadir of IgG between loss of transplacentally acquired maternal IgG and production by the infant. THI is a self-limited disorder and characterized by defect in synthesis of one or more immunoglobulin isotypes in the early years of a child's life. However, it is important to recognize this so that the

Table VIII. Overview of IgG subclass deficiency

IgG1 deficiency

- Associated with hypogammaglobulinemia
- Total IgG is low
- More frequent in adults than children
- Older patients with IgG1 deficiency may evolve into CVID

IgG2 deficiency

- Most common subclass deficiency in children
- Males are more commonly affected than females
- Associated with recurrent or chronic respiratory infections
- Impaired polysaccharide responsiveness

IgG3 deficiency

- Less common than IgG4 and IgG2
- More common in adults
- Associated with other subclass deficiency
- Recurrent infections especially refractory sinusitis and asthma

IgG4 deficiency

- Most common subclass immunodeficiency
- Most are asymptomatic
- Incidence is higher in < 5 years
- Normal antibody response to vaccine antigens
- Often associated with IgG2 and IgA
- Noted in Wiskott- Aldrich syndrome, ataxia-telangiectasia, Muco-cutaneous candidiasis
- Associated syndromes - Down syndrome, Immune thrombocytopenia and Systemic lupus erythematosus

affected patient is not subjected to unnecessary investigations and treatment.The ESID defines THI as reduced IgG, which recovers around by 4th year of age[17], while the WHO/IUIS (World Health Organization / International Union of Immunological Societies) definition mandates reduction of both IgG and IgA.[18] Two distinct clinical presentations can occur in THI: 1. Asymptomatic children screened for significant family history of PID in first and second degree relatives 2. Symptomatic children investigated for PID. Clinical manifestations range from less serious otitis media and upper respiratory infections to severe meningitis and deep tissue infections. In THI, serum IgG values are below the lower limit of normal (less than 2 SD below the mean for a healthy population). THI is a diagnosis of exclusion and goals of management are to ensure prevention and treatment of intercurrent infections. Prophylactic antimicrobials are helpful-amoxicillin (10 mg/kg twice daily). IVIg replacement is occasionally required for recurrent severe infections.[19]

IgG subclass deficiency

IgG subclass deficiency is said to occur when total IgG is normal and at least one of the four IgG subclass level is <2 SD of age specific cut-off. Most IgG subclass deficient patients are asymptomatic. However, patients with IgG2 deficiency can have severe life- threatening infections secondary to encapsulated bacteria and affected individuals have decreased antibody response to polysaccharide vaccines (Table VIII).[20] IgG subclass deficiency is considered clinically significant when patients developed recurrent infections and significant defect in impaired antibody responsiveness. Diagnosis is established by estimation of IgG subclass and comparison with age specific cut-offs. Treatment includes immunization with conjugate vaccines and immunoglobulin replacement therapy in patients with poor response to polysaccharide vaccines and prophylactic antimicrobials for sino-pulmonary infections. Children with IgG subclass deficiency need long-term follow-up.

Specific antibody deficiencies with normal immunoglobulins

Specific antibody deficiency (SAD) is a PID characterized by normal levels of serum immunoglobulins and IgG subclass but with recurrent infection and diminished antibody response to polysaccharide antigens following vaccination. Main clinical manifestations in SAD are otitis media and sino-pulmonary infections. Diagnosis of SAD requires demonstration of poor IgG response to polysaccharide vaccines with normal immunoglobulin levels. SAD severe deficiency shows no protective antibody levels for any serotype. Management includes additional immunization, IVIg replacement and antibiotic prophylaxis and treatment.

Points to Remember

- ***Primary B-cell disorders/ primary antibody deficiency disorders (PADs) are the most common primary immunodeficiency disorders (PID) accounting for approximately 50% of all PID cases.***
- ***Recurrent infections with typical microorganisms and predilection for specific organ systems (sino-pulmonary system, gastrointestinal tract and bloodstream infections) are important clinical pointers to suspect PADs.***
- ***Absent tonsils and non-palpable lymph nodes are simple bedside clues to clinch the diagnosis of X-linked Agammaglobulinemia.***
- ***Compliance with regular intravenous immunoglobulin (IVIg) replacement and prophylactic antimicrobial agents remains the standard of care, with proven benefits in both morbidity and mortality.***
- ***Autoimmunity in the setting of underlying PADs (especially common variable immunodeficiency) has a heterogeneous spectrum of clinical manifestations and needs a high index of clinical suspicion to recognise.***
- ***Appropriate disease specific vaccination plan, genetic counselling and attempts for antenatal diagnosis for monogenic defects are crucial.***

References

1. Abolhassani H, Azizi G, Sharifi L, Yazdani R, Mohsenzadegan M, Delavari S, et al. Global systematic review of primary immunodeficiency registries. Expert Rev Clin Immunol 2020;16(7):717-732. doi: 10.1080/1744666X.2020.1801422. PMID: 32720819.
2. Plebani A, Soresina A, Rondelli R, Amato GM, Azzari C, Cardinale F, et al. Clinical, immunological, and molecular analysis in a large cohort of patients with X-linked agammaglobulinemia: an Italian multicenter study. Clin Immunol 2002; 104(3):221-230. doi: 10.1006/clim.2002.5241. PMID: 12217331.
3. Conley ME, Rohrer J, Minegishi Y. X-linked agammaglobulinemia. Clin Rev Allergy Immunol 2000;19(2):183-204. doi: 10.1385/CRIAI:19:2:183. PMID: 11107501.
4. Conley ME, Howard V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. J Pediatr 2002;141(4):566-571. doi: 10.1067/mpd.2002.127711. PMID: 12378199.

5. Lederman HM, Winkelstein JA. X-linked agammaglobulinemia: an analysis of 96 patients. Medicine (Baltimore) 1985; 64(3):145-156. PMID: 2581110.
6. Ochs HD, Smith CI. X-linked agammaglobulinemia. A clinical and molecular analysis. Medicine (Baltimore) 1996; 75(6):287-299. doi: 10.1097/00005792-199611000-00001. PMID: 8982147.
7. Bonagura VR, Marchlewski R, Cox A, Rosenthal DW. Biologic IgG level in primary immunodeficiency disease: the IgG level that protects against recurrent infection. J Allergy Clin Immunol 2008;122(1):210-212. doi: 10.1016/j.jaci.2008.04.044. PMID: 18602574.
8. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol 1999; 93(3):190-197. doi: 10.1006/clim.1999.4799. PMID: 10600329.
9. Al-Herz W, Bousfiha A, Casanova JL, Chapel H, Conley ME, Cunningham-Rundles C, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. Front Immunol 2011; 2:54. doi: 10.3389/fimmu.2011.00054. PMID: 22566844; PMCID: PMC3342372.
10. Aghamohammadi A, Allahverdi A, Abolhassani H, Moazzami K, Alizadeh H, Gharagozlou M, et al. Comparison of pulmonary diseases in common variable immunodeficiency and X-linked agammaglobulinaemia. Respirology 2010; 15(2):289-295. doi: 10.1111/j.1440-1843.2009.01679.x.Epub 2009 Dec 27. PMID: 20051045.
11. Aghamohammadi A, Abolhassani H, Moazzami K, Parvaneh N, Rezaei N. Correlation between common variable immunodeficiency clinical phenotypes and parental consanguinity in children and adults. J Investig Allergol Clin Immunol 2010; 20(5):372-379. PMID: 20945602.
12. Ogershok PR, Hogan MB, Welch JE, Corder WT, Wilson NW. Spectrum of illness in pediatric common variable immunodeficiency. Ann Allergy Asthma Immunol 2006; 97(5):653-656. doi: 10.1016/S1081-1206(10)61096-4. PMID: 17165275.
13. Douglas SD, Goldberg LS, Fudenberg HH. Clinical, serologic and leukocyte function studies on patients with idiopathic "acquired" agammaglobulinemia and their families. Am J Med 1970; 48(1):48-53. doi: 10.1016/0002-9343(70)90097-5. PMID: 5308993.
14. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International Consensus Document (ICON): Common Variable Immunodeficiency Disorders. J Allergy Clin Immunol Pract 2016; 4(1):38-59. doi: 10.1016/j.jaip.2015.07.025. Epub 2015 Nov 7. PMID: 26563668; PMCID: PMC4869529.
15. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al. The European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis of Inborn Errors of Immunity. J Allergy Clin Immunol Pract 2019; 7(6):1763-1770. doi: 10.1016/j.jaip.2019.02.004. Epub 2019 Feb 15. PMID: 30776527.
16. Albin S, Cunningham-Rundles C. An update on the use of immunoglobulin for the treatment of immunodeficiency disorders. Immunotherapy 2014; 6(10):1113-1126. doi: 10.2217/imt.14.67. PMID: 25428649; PMCID: PMC4324501.
17. Seidel MG, Kindle G, Gathmann B, Quinti I, Buckland M, van Montfrans J, et al.The European Society for Immunodeficiencies (ESID) Registry Working Definitions for the Clinical Diagnosis of Inborn Errors of Immunity. J Allergy Clin Immunol Pract 2019; 7(6):1763-1770. doi: 10.1016/j.jaip.2019.02.004. Epub 2019 Feb 15. PMID: 30776527.
18. Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol 2015; 35(8):696-726. doi: 10.1007/s10875-015-0201-1. Epub 2015 Oct 19. PMID: 26482257; PMCID: PMC4659841
19. Doğu F, Ikincioğullari A, Babacan E. Transient hypogammaglobulinemia of infancy and early childhood: outcome of 30 cases. Turk J Pediatr 2004; 46(2):120-124
20. Morell A. Clinical relevance of IgG subclass deficiencies. Ann Biol Clin (Paris) 1994; 52(1):49-52. PMID: 8210074.

GLOSSARY IN IMMUNOLOGY

Throughput sequencing: (THROO-put) Throughput is the amount of a product or service that a company can produce and deliver to a client within a specified period of time. In medicine, it is used to describe the efficiency of laboratory procedures, such as genetic sequencing, or the number of patients seen in a clinic in a certain period of time.

IMMUNOLOGY

NEUTROPHIL DISORDERS

***Nancy Hilda J**
****Aishwarya Venkataraman**

Abstract: *Neutrophils play an important role in recognition and killing of infectious pathogens. Disorder of neutrophil production, emigration, chemotaxis and function can cause a spectrum of immune defects, which are characterized by recurrent and serious invasive infections. This article is an overview of the common neutrophil disorders.*

Keywords: *Neutrophils, Phagocytes, Neutropenia, Hyper IgE, CGD, Chediak Higashi syndrome.*

Neutrophils - basics

Deriving its name from the staining nature (neutral pink colour on hematoxylin and eosin staining preparation), neutrophil is a type of short- lived white blood cell. Neutrophils are an essential part of innate immunity, the initial response to any recent infection.[1,2] They are one among the first cell types to travel to the site of infection. Like other leucocytes in our immune system, neutrophils play a central role in fighting infection.[1-3]

Morphology: Neutrophils are the most abundant cell type in the blood making 45-75% of the total white blood cells. They are polymorphonuclear (PMN) in nature because of the multi lobed nucleus present in them. The other PMN family cells are basophils and eosinophils. Together they constitute the granulocytic population of leucocytes.

Functions: The functions of neutrophils are multifarious including mechanisms like chemotaxis, phagocytosis, and release of reactive oxygen species (ROS), granular proteins and secretion of cytokines.[1,2] In addition to these well-established mechanisms, neutrophils exhibit 'NETosis', the process of neutrophil extracellular trap (NET) formation which helps in trapping and killing any infectious agent. Although, this recently discovered function of neutrophil is debatable it has gained essential research interest among scientists.[4] Neutrophils are functionally flexible cells highlighting their importance in regulation of immune function. However, neutrophils can play dual role besides their antimicrobial function, deregulation of neutrophils and their hyperactivity during inflammation can lead to tissue damage.[3,5]

* Scientist B

** Scientist E,
ICMR-National Institute for Research in Tuberculosis, Chetpet, Chennai.

email: aishwarya.v@nirt.res.in

Neutrophil disorders

Although neutrophil disorders are rare, they cannot be neglected because of the morbidity/ mortality caused by them.[6,7] Depending on the cause, neutrophil disorders can be classified as the disorders based on

1. Neutrophil count
2. Neutrophil functions
3. Neutrophil degranulation

1) Disorder of neutrophil count

The neutrophil count itself is a determinant of prevailing infection or underlying ailment. Neutropenia is an absolute reduction in the number of circulating neutrophils. After infancy, absolute neutrophil counts (ANC) < 1.5×10^9/litre are considered to be abnormally low and severe infections occur at values below 0.5×10^9/litre. However, reference ranges vary with age and race. Similarly, neutrophil counts obtained many hours after blood collection may show falsely low values.[6,7] The causes of neutropenia can be classified as shown in Table I.

Clinical features: Children with neutropenia commonly present with malaise and lethargy (parents often describe their children as totally unwilling to get up and walk around), skin infections (cellulitis and subcutaneous abscesses), mucosal and respiratory infections (gingivitis, stomatitis, apthous ulcers, periodontitis, perirectal abscess, pneumonia, and otitis media) and septicaemia.

Diagnosing neutropenia: The urgency of investigations depends on age and clinical presentation. Asymptomatic children with isolated neutropenia can be observed for several weeks, whereas those in whom the history and examination findings suggest the possibility of a serious underlying problem need prompt evaluation.[7,8]

The following tests[7,8] should be considered. (1) Repeated full blood counts will help distinguish transient, cyclical and chronic neutropenias. This will also help differentiate isolated neutropenia from that associated with immunological or oncological disease. (2) Neutrophil antibody assays are usually performed and are positive in antibody mediated neutropenias. (3) Bone marrow aspiration is useful to rule out aplastic anaemia, leukaemia, and other malignancies. (4) Serum immunoglobulin estimations are an essential investigation in patients with neutropenia. Persistent or cyclic neutropenia is associated with hyperIgM syndrome (CD40 ligand or gp39 deficiency). Neutropenia is also noted in boys with X linked agammaglobulinaemia (XLA). (5) In addition, lymphocyte subsets should be analysed to exclude reticular dysgenesis in infants. An autoantibody screen should be done to rule out systemic lupus erythematosus and other autoimmune conditions.

Treatment: Management depends on the cause, severity and clinical course of neutropenia. General measures to prevent and treat infections is essential in all cases of chronic neutropenia. Prompt recognition of serious infection and, early administration of parenteral broad-spectrum antibiotics is essential. Regular prophylactic cotrimoxazole can be used in children with chronic neutropenias. Several studies have shown beneficial effect of granulocyte colony-stimulating factor (rhG-CSF)[7-9] on the neutrophil count in children with resolution of pre-existing infections, reduced numbers of new infections and significant improvement in survival and quality of life. The dose required to maintain ANC over 1.0×10^9 /litre ranged from 0.8-60μg/kg/ day. Intravenous immunoglobulin treatment (IVIG) is useful in antibody mediated neutropenia. However, the effect of IVIG is short lived and this should be used occasionally. Granulocyte or buffy coat transfusions can also be used as an adjuvant therapy in children with chronic neutropenia and focal bacterial or fungal infections not responsive to regular treatment. Parenteral neutrophil transfusions have been described in the treatment of neonates with alloimmune neutropenia but, the safety and effectiveness are unknown. Bone marrow transplantation (BMT) can be considered for those who do not respond to rhG-CSF.[1,7,8]

2) Disorders of neutrophil function

a. Chronic granulomatous disease (CGD): This is a rare disorder characterised by absent or reduced function of the respiratory burst, the process that produces oxygen free radicals important for intracellular killing. CGD is caused by congenital defects in the five components of the enzyme NADPH oxidase. CGD occurs as an X linked recessive

Table I. Causes of neutropenia

1. Transient
2. Chronic i. Congenital neutropenias a. Failure of production (bone marrow) b. Severe chronic neutropenia c. Cyclic neutropenia d. Reticular dysgenesis ii. Associated with syndromes a. Primary immunodeficiencies: XLA, hyper-IgM b. Glycogen storage disease (1b) c. Shwachman diamond syndrome d. Cardioskeletal myopathy e. Onychotrichodysplasia
3. Antibody mediated neutropenias a. Autoimmune neutropenia of infancy b. Alloimmune neonatal neutropenia c. Neonatal neutropenia owing to autoimmune disease in mother d. Autoimmune neutropenia seen in association with primary specific immunodeficiencies
4. Idiopathic e. Chronic benign neutropenia

XLA, X linked agammaglobulinaemia

disease caused by a mutation in the glycoprotein 91 phagocyte oxidase gene (gp91 phox gene), as well as in an autosomal recessive manner and are caused by defects in the cytosol components p47phox (25%), p67phox (5%), or the smaller membrane bound subunit, p22phox (5%).[7, 10-13]

Clinical features: Most children with CGD present in the 1st year of life with recurrent bacterial and fungal infections. The most commonly seen pathogens are *S aureus, Aspergillus spp*, enteric gram negative bacteria and *Burkholderia cepacia*.[10,13] Infections include pneumonia, cutaneous infections (including perirectal abscesses), lymphadenitis, liver abscess, osteomyelitis, septicaemia and otitis media. As most children have the presence of granulomas caused by the chronic inflammatory response to the pathogen, CGD can mimic Crohn's disease or can present with obstructive gastrointestinal symptoms.

Investigations: The diagnosis of CGD can be made by the bacterial killing test, which is a screening test for defects in opsonisation, phagocytosis, or intracellular killing. It looks at the ability of phagocytes to kill catalase positive bacteria, such as *S aureus*, in vitro. The phorbolmyristate acetate (PMA) nitroblue tetrazolium (NBT) slide test is an alternative screening test for disorders of oxidative metabolism.[7,10,13] More sensitive quantitative tests to measure respiratory burst activity in stimulated neutrophils using dihydrorhodamine(DHR) flow cytometric analysis are now available. Prenatal diagnosis of CGD can be made by the analysis of DNA from chorionic villous sampling or amniotic fluid cells if the specific mutation in the family has previously been identified.

Treatment: Treatment involves measures to reduce the frequency of infections and to ensure their prompt recognition and treatment. Live bacterial vaccines like BCG is contraindicated; however, children should receive all other routine immunisations. Regular prophylaxis with cotrimoxazole can reduce bacterial infections. Similarly, antifungal prophylaxis with itraconazolecan be used but should be reserved for severe cases or where invasive fungal infection has previously occurred. Bone marrow transplantation using matched siblings as donors have been reported with successful outcomes.[7,13]

b. Other disorders of intracellular killing

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency
- Glutathione synthetase deficiency
- Myeloperoxidase (MPO) deficiency

c. Leukocyte adhesion deficiency: Leukocyte adhesion deficiency (LAD) is characterized by an inability of leukocytes to migrate to the site of infection to kill offending microbes. There are three different types of LAD:

1. Type I - in which steady adhesion of leukocyte to endothelial surfaces is defective by mutations in CD18 gene resulting in defective or deficient beta-2 integrin

2. Type II - in which there is an absence of Sialyl Lewis X of E-selectin

3. Type III - in which there is a defect in beta integrins 1, 2, and 3; this impairs the integrin activation cascade - specifically, a mutation in the kindlin-3 gene causes this type of LAD

Characteristics of LAD include

LAD I

- Delayed separation of the umbilical cord
- Recurrent pyogenic infections, with onset in the first weeks of life
- Infections caused meanly by Staphylococcus aureus and Pseudomonas aeruginosa
- Absent pus formation
- Periodontitis

LAD II

- Recurrent skin infections
- Pneumonia
- Bronchiectasis
- Tuberculosis
- Denture abnormalities
- Infections are less severe and fewer as compared to LAD I

LAD III

- Omphalitis
- Osteoporosis like bone features
- Bleeding complications
- Hematological abnormalities, e.g., bone marrow failure

Other manifestations of LAD

- Infections like perianal abscess, osteomyelitis, necrotic soft tissue infection, otitis media, meningitis, granuloma, pyoderma gangrenosum, oral candidiasis, and severe gingivitis.

3) Other disorders of chemotaxis

Normal chemotaxis is essential for the movement of neutrophils from the circulation to the site of infection and requires the generation of chemotactic substances. Abnormal neutrophil chemotaxis is seen in a various conditions; most important being Hyper-IgE syndrome.

Hyper-IgE syndrome

This presents in infancy with serious recurrent staphylococcal infections of the skin and the lungs, which sometimes develop into cystic pneumonias. The children may have coarse facies and chronic eczematoid dermatitis. The condition can occur sporadically or as an autosomal dominant condition. Although the cause of infections is not clearly understood, it is thought to be due to the presence of high concentrations of antistaphylococcal IgE, low concentrations of antistaphylococcal IgG and/or a neutrophil chemotactic defect. It has been noted that lymphocytes of children with hyper-IgE syndrome have an impaired response to IL-12, leading to decreased IFN-γ production. Thus, it has been suggested that a defective IL-12–IFN-γ pathway plays an important role in the pathogenesis of the hyper-IgE syndrome.[7,14,15] Diagnosis is always a challenge as it is very difficult to differentiate between children with Hyper IgE and severe atopic eczema with associated staphylococcal infection.

Investigations: Blood and sputum examination may show eosinophilia and raised serum IgE, often more than 5000 IU/litre. Lymphocyte counts are normal. Elevated IgDlevels and normal IgG, IgA and IgM are noted in Hyper IgE syndrome.

Treatment: Prophylactic antibiotics are the mainstay of the management. Intravenous immunoglobulin can be used for those who are deficient in immunoglobulins or IgG subclasses, or have a known defect in antibody responses to polysaccharide. Recently, IFN-γ have been used with improvement in neutrophil chemotaxis.[7,16]

4) Disorders of degranulation: The two main conditions associated with granulation defects are Chediak Higashi syndrome (CHS) and specific granule deficiency.[17,18]

a. Chediak Higashi syndrome (CHS) is a rare autosomal recessive disorder of granule bearing cells. Children with CHS present in childhood with partial albinism (hypopigmentation of hair and eyes compared with other family members) and recurrent infections of skin, mouth, and respiratory tract. Death often occurs before 7 years of age because of serious infections. In the accelerated phase many children present with hemophagocytic lymphohistiocytosis. Children who survive to become adults may have neurological disabilities. The peripheral smear shows large cytoplasmic granules in neutrophils and lymphocytes. Tests for neutrophil function show defective chemotaxis and intracellular killing. Lymphocyte function and platelet aggregation are also abnormal. Treatment involves prompt recognition and treatment of infections. Bone marrow transplantation done with HLA matched marrow before the full blown accelerated phase, has been successful.[7,16,18,19]

b. Specific granule deficiency: This is an extremely rare, autosomal recessively inherited condition characterised by recurrent bacterial infections starting in infancy associated with absent granules in peripheral blood neutrophils. Management consists of prophylactic antibiotics and aggressive management of infections; individuals have survived into adulthood.

Approach to children suspected to have neutrophil disorders

Children with chronic or recurrent infections and those who have a slow response to antibiotics must be evaluated neutrophildisorders. Some of the investigations are as follows:[2,6,7,16]

a. A full blood count and peripheral smear examination

b. The NBT test and/or quantitative tests to measure respiratory burst activity in stimulated neutrophils using flow cytometric analysis if CGD is suspected.

c. Measurement of G6PD and glutathione synthetase.

d. CD11/CD18 expression in peripheral blood neutrophils to diagnose LAD 1.

e. Flow cytometric analysis for SLeX (CD15) expression on leucocytes using an anti-SLeX monoclonal antibody for LAD 2.

f. Estimation of serum immunoglobulin isotypes, mannan binding lectin, and total complement haemolytic activity and DNA studies for Fcγ receptor polymorphisms.

Further detailed investigations are difficult to perform and many of these tests are not often performed outside a research setting. However, recent studies have described neutrophils as being used as biomarkers to diagnose and treat certain conditions.

Neutrophil extracellular traps as biomarkers

Apart from harbouring important immune functions, neutrophils can also be used as biomarkers to diagnose and treat certain disorders including autoimmune disorders. Neutrophils, as a 'do or die' phenomenon, extrude a web of DNA-rich material covered with antimicrobials that entrap and subsequently kill microbes appropriately, called neutrophil extracellular traps (NETs).[4] Quantifying NETs is now being seen as a tool in diagnosing and/or assessing disease outcome. This is done by estimating NET markers like cell-free DNA (cfDNA), DNA complexes with myeloperoxidase (MPO- DNA) , reactive oxygen species (ROS), elastase etc. For example, studies have identified that the deposition of NETs observed in various inflammatory pathologies is associated with the circulating cell-free DNA (cfDNA) levels in biological fluids (plasma and serum) of the respective patients. Thus, it is understood that even though they play a key role in defense against pathogens, NETs may also cause undesirable effects to the host. Most importantly, in the prevailing pandemic Corona virus disease- 19 (COVID- 19) ROS-NET pathway is proven to play a role in thrombosis formation.

Conclusion

Neutrophil disorders are a rare, but important reason of morbidity and mortality in infants and children. Neutrophil disordersshould be considered when a child presents with serious or recurrent infections and in those who are being investigated for immunodeficiency. High index of suspicion is required especially when a child presents with history of oral ulcers and gingivitis, delayed separation of the umbilical cord, skin abscesses, perianal and perirectal abscesses, poor wound healing, sinopulmonary infections, or deep visceral abscesses presence of uncommon organisms such as S marcescens or Pseudomonas spp. It should also be suspected in a child with syndromic features associated with neutropenia. Appropriate and prompt investigations can lead to definite diagnoses and specific management measures can reduce both mortality and morbidity.

Points to Remember

- ***Neutrophil disorders are a rare, but important reason of morbidity and mortality in infants and children.***
- ***Neutrophil disorders should be considered when a child presents with serious or recurrent infections and in those who are being investigated for immunodeficiency.***
- ***Appropriate and prompt investigations can lead to definite diagnoses, and specific management measures can reduce both mortality and morbidity.***

References

1. Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM, Chilvers ER. Neutrophil kinetics in health and disease. Trends Immunol 2010; 31:318-324.
2. Rosales C. Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? Front Physiol 2018; 9:113. doi.org/10.3389/fphys.2018.00113
3. Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. Annu Rev Pathol 2014; 9: 181-218.
4. Giaglis S, Hahn S, Hasler P. "The NET Outcome": Are Neutrophil Extracellular Traps of Any Relevance to the Pathophysiology of Autoimmune Disorders in Childhood? Front Pediatr 2016; 4:97.
5. Kobayashi SD, Malachowa N, DeLeo FR. Neutrophils and Bacterial Immune Evasion. J Innate Immun 2018; 10: 432-441.
6. Dinauer MC. Disorders of neutrophil function: an overview. Methods Mol Biol 2007; 412:489-504.
7. Lakshman R, Finn A. Neutrophil disorders and their management. J Clin Pathol 2001; 54:7-19.
8. Bernini JC. Diagnosis and management of chronic neutropenia during childhood. Pediatr Clin North Am 1996; 43:773-792.
9. Rebuck N, Finn A. Polymorphonuclear granulocyte expression of CD11a/CD18, CD11b/CD18 and L-selectin in normal individuals. FEMS Immunol Med Microbiol 1994; 8:189-195.
10. Roos D. Chronic granulomatous disease. Br Med Bull 2016; 118:50-63.
11. Mills EL, Quie PG. Congenital disorders of the function of polymorphonuclear neutrophils. Rev Infect Dis 1980; 2:505-517.
12. Weening RS, Adriaansz LH, Weemaes CM, Lutter R, Roos D. Clinical differences in chronic granulomatous disease in patients with cytochrome b-negative or cytochrome b-positive neutrophils. J Pediatr 1985; 107: 102-104.
13. Forrest CB, Forehand JR, Axtell RA, Roberts RL, Johnston Jr RB. Clinical features and current management of chronic granulomatous disease. Hematol Oncol Clin North Am 1988; 2:253-266.
14. Borges WG, Augustine NH, Hill HR. Defective interleukin-12/interferon-gamma pathway in patients with hyperimmunoglobulinemia E syndrome. J Pediatr 2000; 136:176-180.

15. Grimbacher B, Holland SM, Gallin JI, Greenberg F, Hill SC, Malech HL, et al. Hyper-IgE syndrome with recurrent infections-an autosomal dominant multisystem disorder. N Engl J Med 1999; 340:692-702.
16. Kyono W, Coates TD. A practical approach to neutrophil disorders. Pediatr Clin North Am 2002; 49:929-971, viii.
17. Barrat FJ, Auloge L, Pastural E, Lagelouse RD, Vilmer E, Cant AJ, et al. Genetic and physical mapping of the Chediak-Higashi syndrome on chromosome 1q42-43. Am J Hum Genet 1996; 59:625-632.
18. Kaplan J, De Domenico I, Ward DM. Chediak-Higashi syndrome. Curr Opin Hematol 2008; 15:22-29.
19. Haddad E, Le Deist F, Blanche S, Benkerrou M, Rohrlich P, Vilmer E, et al. Treatment of Chediak-Higashi syndrome by allogenic bone marrow transplantation: report of 10 cases. Blood 1995; 85:3328-3333.

CLIPPINGS

Risk factors for acquisition of scrub typhus in children.

Scrub typhus is a mite borne zoonosis common in the tropics affecting children with no good preventive strategy leading to considerable morbidity and mortality.

A case control study with a 1:2 case control ratio was conducted over a 2year period at a tertiary care centre and its surrounding districts in South India. Cases were children <15years with confirmed scrub typhus. Controls were age and locality matched community controls without fever. Demographic, environmental and behavioral risk factors were obtained in cases and controls by an interview and an environmental survey. A vector survey was also undertaken in the immediate vicinity of the cases.

101 cases and 167 controls were analyzed. On multivariate analysis, significant association was observed with environmental factors such as the presence of a water body within 100m of the house (OR 3.56(1.36, 9.75); p 0.011), cooking outside the house (OR 5.61 (1.51, 23.01); p 0.011), owning pets (OR 3.33(1.16, 9.09); p 0.031), and the presence of bushes within 5m of the house (OR 2.78 (1.11, 7.69); p 0.033). Of the behavioral factors, the child going to school by a vehicle (OR 3.12 (2.29, 8.37); p 0.006) was associated with an increased risk. Drying clothes on a clothesline showed a trend towards protection from acquiring scrub typhus (OR 0.31 (0.08, 1.08); p 0.077).

Vector survey: 26 rodents were trapped in many houses. Trombiculid mites were isolated in 24 houses with 9(34.6%) being able to transmit scrub typhus. 254 trombiculid mites belonging to four species and two genera were collected. *Leptotrombidiumdeliense,* (33.5%). *Schoengastiella ligula,* (11.0%) of the total mite specimens collected. *S. ligula* always co-existed with*L.deliense.* The estimated Chigger index for *Leptotrombidiumdeliense* and *Schoengastiella ligula* was 3.27and 1.08 per animal respectively.

A clean peri-domestic environment free of vegetation, drying clothes on a clothesline and cooking indoors may decrease the risk of scrub typhus.

Rose W, Kang G, Verghese VP, Candassamy S, Samuel P, Prakash JJA, et al.Risk factors for acquisition of scrub typhus in children admitted to a tertiary centre and its surrounding districts in South India: a case control study.BMC Infect Dis 2019; 19:: 31349809665.Published online 2019 Jul 26. doi: 10.1186/s12879-019-4299-2.PMCID: PMC6660696.PMID.

GLOSSARY IN IMMUNOLOGY

High throughput sequencing: Sequencing information has traditionally been elucidated using a low throughput technique called Sanger sequencing, high throughput sequencing (HTS) technologies are capable of sequencing multiple DNA molecules in parallel, enabling hundreds of millions of DNA molecules to be sequenced at a time.

IMMUNOLOGY

COMPLEMENT DEFICIENCY IN SYSTEMIC AUTOIMMUNE DISEASES

***Madhubala Sharma**
****Sumit Goel**
*****Aaqib Zaffar Banday**
******Amit Rawat**

Abstract: *Complement is an important part of the innate immune pathway. It involves over 20 serum proteins, most being synthesized in liver. These proteins are initially inactive precursors which get activated later by different stimuli. All the three pathways of complement activation i.e., classical, alternative and lectin converge to produce membrane attack complex or terminal complex which leads to lysis of the target pathogen. Activity of complement is controlled by regulatory proteins that prevent host cell damage and lysis caused by inadvertent binding of activated complements. Complement deficiency results in autoimmune diseases. Early complement deficiency results in monogenic lupus and infections due to encapsulated bacteria whereas late complement component deficiency causes neisserial infections. Complements can be assessed by various tools like enzyme-linked immunoassays, flow cytometry, and next-generation sequencing.*

Keywords: *Early-onset systemic lupus erythematosus, Complement, Classical pathway, Autoimmune diseases, Atypical hemolytic uremic syndrome, Alternative pathway.*

The human immune system comprises two major arms i.e., innate and adaptive. Components of the innate immune system, elements of which are present in lower animals and even plants, do not require prior priming and are already geared for a rapid immune response, unlike the adaptive immune response which takes time to kick in. The complement system is an important component of the innate immune armament. It is made up of plasma proteins that are activated by pathogens directly or indirectly by pathogen-bound antibodies (classical pathway), which leads to a cascade of reactions occurring on the surface of pathogens leading to lysis of the pathogens. Activity of the complement is controlled by regulatory proteins that prevent host cell damage and lysis caused by inadvertent binding of activated complements. It consists of effector proteins and receptor molecules that provide both protection against pathogens and regulation of immune response. In 1899, Paul Ehrlich coined the term 'complements' meaning heat-labile molecules in serum that provide anti-microbial immunity in conjunction with antibodies.[1] Later several components of the complement cascade were described and numbered. We now know that the complement system comprises around 50 serum and membrane-bound proteins that regulate a proteolytic activation cascade, culminating in production of effector molecules with multiple functions.[2] However, complement activation is also intimately involved in the pathogenesis of several systemic autoimmune diseases. Excessive complement activation is one of the major reasons for tissue injury and end-organ damage in autoimmune diseases.[3] Contradictorily, deficiency of some of the complement components results in autoimmune diseases like systemic lupus erythematosus (SLE).[4] Our current review focuses on monogenic defects of complement components and their impact on predisposition to infections and autoimmunity.

* Ph.D., Scholar
** Senior Research Fellow
*** Senior Resident
**** Professor,
Allergy Immunology Unit, Department of Pediatrics,
Advanced Pediatrics Centre,
Postgraduate Institute of Medical Education
and Research, Chandigarh.
email: rawatamit@yahoo.com

Three major pathways of complement pathways: Complement activation include serum proteins such as C1q, C1r, C1s, C2 to C9, factor B, and properdin. There are three main complement activation pathways - classical, alternative, and lectin pathway. All these three pathways converge leading to activation of C3 and C5 resulting in the formation of membrane attack complex (MAC or C5b-C9). MAC form pores on the target membrane thereby disrupting it and causing cell lysis. Three different complement pathways get activated via proteolysis of zymogens or precursors present in circulation (Fig.1).

a) The classical pathway is mainly activated by IgG or IgM immune complex deposits. During antigen-

antibody interaction, complement binding site present on Fc portion of the antibody gets exposed which binds to C1q, initiating the assembly and activation of multimolecular C1 complex, (C1q-$C1r_2$-$C1s_2$). Further, C1s activates C4 and C2 leading to the formation of C3 convertase (C4b2a). C3 convertase further forms activated C3 (C3b) and leads to the formation of C5 convertase (C3bBbC3b) that subsequently creates MAC on the target membrane.

b) The second pathway is antibody independent and is called the 'alternative pathway' (AP). Specific proteins involved in AP include factor B, factor D, factor H, and factor P (properdin). AP is initiated by a tick-over mechanism - a small portion of C3 in circulation is constantly hydrolyzed by water forming C3(H_2O). It binds to factor B, which is activated by factor D and forms C3(H_2O)Bb called C3 convertase. It is relatively labile and initiates C3 cleavage. Properdin stabilizes the short-lived C3 convertase and forms C5 convertase (C3bBbP). It activates C5 forming C5b and further supports the formation of MAC.

c) The third pathway, also known as the 'lectin pathway', is initiated by the binding of particular pattern-recognition moieties like mannan-binding lectins (MBL) or ficolins to bacterial membranes. Further, MBL-associated serine protease activates C2 and C4, initiating the cascade and terminating in the formation of MAC complex.[5, 6]

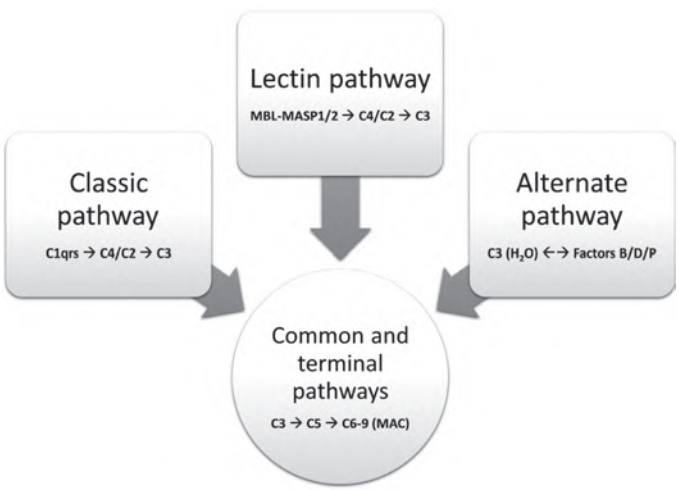


Fig.1. Complement activation pathways

MBL: Mannose-binding lectin, also known as mannan-binding protein (MBP); MASP: MBL-associated serine protease; MAC: membrane attack complex (MAC), or terminal complement complex.

Complement deficiency in autoimmune diseases: Important mechanisms involved in the pathogenesis include aberrant complement regulation and poor clearance of apoptotic debris that may stimulate the presentation of altered self-antigens. Complement components also assist in the disposal of immune complexes, inappropriate functions of which cause tissue injury and production of inflammatory cytokines. Circulating autoantibodies against complement proteins, like C1q and C3b, tend to get deposited in the kidney causing inflammation and tissue injury resulting in lupus nephritis-like phenotype.[7]

Deficiency of early complement components is, by far, the most important cause of 'monogenic lupus' - SLE-like manifestations (often accompanied by recurrent/persistent infections) resulting from single-gene variants that follow a Mendelian pattern of inheritance. Monogenic lupus often presents in the first decade of life and, hence, is an important consideration in children who present with SLE-like manifestations in early childhood (termed as 'early onset' SLE). In 1970, the first familial case of early-onset SLE was described in children due to C1 deficiency. Subsequently, many classical pathway components like C1q, C1r, C1s, C2, C3 and C4 have been associated with inherited complement deficiencies, with lupus-like manifestations.[7-10] The epidemiology, clinico-laboratory profile, and underlying molecular defects in

Box 1. Unusual or atypical features of SLE that raise the suspicion of complement deficiency lupus

- Early age of onset.
- Presence of a significant family history.
- Male gender.
- History of recurrent, persistent, or unusually complicated infections; especially, preceding immunosuppressive therapy.
- Predominance of neurological manifestations.
- Speckled pattern of antinuclear antibody (ANA) positivity on indirect immunofluorescence.
- Negative or low-titers of anti-double-stranded deoxyribonucleic acid (anti-dsDNA) antibody even in presence of active lupus.
- Normal levels of C3 and C4 in presence of active disease.
- Undetectable or very low levels of a single complement factor, for example, C3, C4, C2, or C4.
- Undetectable to very low (close to 0) AH50 or CH50.

Table I. Complement deficiencies of classical, alternate, and terminal pathways, associated clinical pathology with genetic deficiency, disease prevalence, chromosomal location of implicated genes and their mode of inheritance

Complement deficiency	Pathology	Gene	Inheritance	Locus	Prevalence	OMIM number
C1q	Nephritis, CNS involvement, photosensitivity, Rash, Encapsulated bacterial infections	C1QA C1QB C1QC	AR	1p36.3-p34.1	~80 cases	120550, 601269, 120575
C1r	Glomerulonephritis, Encapsulated bacterial infections	C1S	AR	12p13	<20	216950
C1s	Nephritis, Encapsulated bacterial infections	C1R	AR	12p13	<20	120580
C2	Malar rash, Discoid rash, serositis, photosensitivity and articular involvement, mild or absent renal, neurological or pleuropericardial involvement, Encapsulated bacteria, Pyogenic infections, Streptococcus pneumoniae sepsis, meningitis	C2	AR	6p21.3	~1:10,000	217000
C3	Malar rash, photosensitivity, arthralgia, Raynaud's phenomenon, Recurrent pyogenic infections	C3	AR	19p13,	<50 cases	120700
C4	Multiorgan involvement, Glomerulonephritis, Encapsulated bacterial infections	C4	AR	6p21.3	~30 cases	120810
C5	Multiorgan involvement, Neisserial Infections	C5	AR	9p34.1	~50 cases	120900
C6	Multiorgan involvement, Neisserial Infections	C6	AR	5p13	1/2,000 in Afro-American population Extremely rare elsewhere	217050
C7	Multiorgan involvement, Neisserial Infections	C7	AR	5p13	<100 cases	217070
C8a C8b	Multiorgan involvement, Neisserial Infections	C8A C8B	AR AR	1p32 1p32	<100 cases	120950 120960
C9	Multiorgan involvement, Neisserial Infections	C9	AR	5p13	1/1000 in Japanese population Extremely rare elsewhere	120940

Complement deficiency	Pathology	Gene	Inheritance	Locus	Prevalence	OMIM number
Factor H	Renal Disease (C3G and aHUS) bacterial infection AMD, SLE	CFH	AR	1q32	<200 cases	134370
Factor I	Bacterial infection Renal diseases (MPGN-II & aHUS)	CFI	AR	4q25	<100 cases	217030
C1 INH	HAE	SERPING1	AD	11q12	1:10,000-1:50,000	606860
Factor D	Bacterial infections	CFD	AR	19p13	3 cases	134350
Factor B	Neisseria meningitis infections	CFB	AR	6p21	2 cases	138470
Properdin	Severe Neisserial infections	CFP	XL	Xp11	100-500 cases	300383

AR- Autosomal Recessive, AD -Autosomal Dominant, XL- X-linked, CNS- Central Nervous system, C3G-C3 Glomerulopathies, AMD- Age-related Macular Degeneration, aHUS- atypical HaemolyticUraemic syndrome, MPGN-II- Membranoproliferative Glomerulonephritis Type II, HAE-Hereditary Angioedema

Table II. Laboratory assessment for characterisation of complement deficiencies

Complement Deficiency	Classical pathway activity (CH50)	Alternative pathway activity (AH50)	Mannan Binding Lectin Pathway activity	ANA	dsDNA	C3	C4
C1q	Reduced	Normal	Normal	Positive (speckled pattern)	Usually, negative	Normal	Normal
C1r	Reduced	Normal	Normal	Positive	Usually, negative	Elevated	Elevated
C1s	Reduced	Normal	Normal	Positive	Positive/ Negative	Elevated	Elevated
C2	Reduced	Normal	Reduced	Positive	Positive/ Negative	Normal	Normal
C3	Reduced	Reduced	Reduced	Positive/ Negative	Positive/ Negative	Reduced	Normal
C4	Reduced	Normal	Reduced	Positive	Positive/ Negative	Normal	Reduced
C5-C9	Reduced	Reduced	Reduced	-	Positive/ Negative	Normal	Normal
Factor H	Reduced	Reduced	Reduced	-	-	Reduced	Normal
Factor I	Reduced	Reduced	Reduced	-	-	Reduced	Normal
C1 INH	Reduced	Normal	Reduced	-	-	Normal	Reduced
Factor D	Normal	Reduced	Normal	-	-	Normal	Normal
Factor B	Normal	Reduced	Normal	-	-	Reduced	Normal
Properdin	Normal	Reduced	Normal	-	-	Normal	Normal

Box 2. Usual or typical features of SLE

- Peak incidence in adolescence
- Female preponderance (Female to male ratio of upto 9-10 to 1 in the adolescent age group)
- Lack of a conspicuous family history
- Infections usually occur after initiation of immunosuppressive therapy
- Predominance of non-neurological (example, hematological, renal and musculoskeletal) manifestations
- Homogenous, often with rim enhancement, pattern of ANA positivity on indirect immunofluorescence
- Elevated titers of (anti-dsDNA) antibody especially in presence of active lupus
- Proportionately decreased levels of C3 and C4 in presence of active disease
- Proportionately low levels of early complement factors, for example, C1q and C2, during active disease (due to consumption)
- Low AH50 and/or CH50 (well above 0) in presence of disease activity

complement deficiencies are summarized in Table I and II. Concisely, it is prudent to consider and evaluate for inherited complement deficiency in all children and adolescents who present with lupus-like manifestations and have one or more of the clinical features described in Box 1 (Based on the profile summarized in Table I and II).

It is crucial to contrast the above-mentioned features of complement-deficiency lupus with the usual clinical presentation of pediatric SLE (non-complement deficiency) that usually manifests as follows, which are given in Box 2.

Thus, complement deficiency lupus may have a distinctive clinical feature as compared to routinely (non-complement deficiency) seen clinical presentation of pediatric SLE. However, the presence of anti-C1q (or other anti-complement) antibodies, which may be seen in a significant subset of 'routine' SLE patients, may mimic inherited complement deficiency lupus. Specific anti-complement antibody testing and genetic evaluation are imperative to diagnose monogenic disease in such conditions.

Specific monogenic complement deficiency disorders are briefly described below:

1. **C1q Deficiency:** C1q deficiency is, by far, the most important inherited complement deficiency which results in SLE-like manifestations in a majority of C1q-deficient individuals. Unlike other complement components which are known to produce in the liver; C1q is produced by bone-marrow-derived monocytes (therefore amenable to treatment with hematopoietic stem cell transplant). C1q is also unique amongst other complement proteins given its critical structural and functional properties. Three different genes encode for C1q protein which consists of 18 polypeptide chains.[6]In addition to its crucial role in the clearance of immune complexes and apoptotic bodies, C1q also plays a critical role in the regulation, maturation, and functioning of dendritic cells. C1q receptors present on monocytes have been reported to act as sensors for membrane-bound C1q and contribute to the switching of monocytes to dendritic cells or macrophages. Regulation of immune-complex mediated type-1 interferon production from plasmacytoid dendritic cells and modulation of effector CD8 T cell responses are its additional functions.[11]

   More than 80 cases of homozygous C1q deficiency have been reported.[3, 5]Although the clinical presentation in these patients may vary, common manifestations include photosensitivity and discoid lupus-like lesions, nephritis, CNS disorders, oral ulcers, arthritis, and recurrent potentially life-threatening bacterial infections. Speckled pattern of ANA positivity mainly results due to the presence of anti-Ro/SSA antibodies in these individuals.[12, 13]

2. **C1s and C1r deficiency:** C1s and C1r are paralogous proteins that exist as a tetrameric structure (C1s-C1r-C1r-C1s) in the circulation and form a multi-molecular C1complex along with C1q.

   Similar to C1q deficiency, most of the C1s and C1r deficient patients develop SLE. However, deficiency of these complements is much rarer (about two C1r and seven C1s genetically confirmed patients). Besides, they present with recurrent bacterial, viral, or fungal infections, severe cutaneous disorders, and renal disease.[14,15]

3. **C2 deficiency:** C2 deficiency is the most common complement deficiency among Caucasians. There are two types of C2 deficiency: Type 1 is the most common (90%) resulting from absent protein synthesis; whereas, in Type II C2 deficiency, protein is synthesized but not secreted. Only ~10% of C2 deficient patients develop SLE.[16, 17]

4. **C3 deficiency:** C3 is the most abundant complement in the peripheral circulation and is central to all complement pathways. C3 deficient individuals present with cutaneous disorders, severe glomerulonephritis, chronic lung disease, polyarthritis, and recurrent infections .[18]

5. **C4 deficiency:** *C4* gene, present in HLA class III region, is notable for its copy number variations - two to eight copies of *C4* gene are normally present in a diploid genome. Each *C4* gene either codes for C4A or C4B protein. Complete *C4* deficiency is rare but deficiency of C4A or C4B is commonly seen in autoimmune diseases and has been associated with the development of juvenile idiopathic arthritis, vasculitis illness like glomerulonephritis, Henoch-Schonlein purpura, and Wegener's granulomatosis in addition to SLE. Failure to achieve normal C4 levels in SLE patients during disease remission is an important clinical pointer towards the presence of a significant copy number variation.[19]

6. **C5-C9 deficiency:** The components of the complement system including C5, C6, C7, C8, and C9 are known as MAC components or terminal complement complex (TCC) or late complement pathway components. C6, C7, and C9 are not only structurally and biochemically similar but also share the functional similarities being the members of the same functional unit. TCC (C6-C9) are activated sequentially after C5 cleavage by C5 convertase (either classical or alternative).[20] Since TCC is an integral part of MAC, deficiency of its components leads to impaired bactericidal activity against Gram-negative infections, especially *Neisseria* sp.[21-23]

7. **Properdin deficiency:** Properdin is a salient positive regulator of alternate pathway (AP) that escalates the half-life of C3 and C5 convertase.[24] Properdin deficiency is X-linked resulting in a predisposition to developing encapsulated bacterial infections, most notably with *Neisseria* sp.[25] Interestingly, the generation of functionally inactive MAC due to TCC deficiency may be beneficial in autoimmune diseases.[26]

Deficiency of complement regulators and receptors: The complement regulators include CR1, CR2, C4-binding protein, decay accelerating factor (DAF), MCP, complement factor H-related proteins, and factor H and are also called the "C3b binding protein family". The key function of regulatory proteins is to protect host cells against the over-activated complement system by C3b inactivation. Genetic deficiency of factor I, which allows unbridled activation of AP and depression of both C3 component and factor B levels, has a low frequency. Patients with insufficient factor I, as in factor H deficiency, usually present with bacterial infections. Deficiency in regulator proteins CD59 and DAF, due to somatic mutations resulting in defective glycophosphatidyl inositol (GPI) anchor synthesis, result in paroxysmal nocturnal hemoglobinuria (PNH) - a disorder characterized by enhanced sensitivity of erythrocytes to complement-mediated lysis. Clinically, DAF deficiency may not be obvious, since its activities are mediated by other molecules, such as CR1 and factor H, as well. Deficiency of CR1 has been correlated with mesangiocapillary glomerulonephritis. Factor H deficiency can present as atypical hemolytic uremic syndrome (aHUS) which can be either be sporadic or familial. Excessive activation and/or dysregulation of AP play a crucial role in the disease pathogenesis in both sporadic and familial aHUS. Loss-of-function mutations in genes encoding the complement regulators (membrane cofactor protein/CD46 and factor I) and gain-of-function mutations in the complement activator genes have also been described.[27]

Laboratory assessment of complement deficiency

Some basic laboratory evaluations for complement deficiency include serum C3 and C4 levels. Patient serum/plasma sample should be immediately stored at -80°C after separation to prevent in-vitro complement activation. Complement components such as C1q, C1r, C1s, C2, C3, C4, and other complement split products can be measured using multiple techniques including nephelometry, ELISA, multiplex bead-based suspension arrays, radial Immunodiffusion, Western blot, etc. ELISA can also test individual complement regulatory factors like factor B, factor H, factor I, factor D and split products of complement pathways. Evaluation of function of complement pathways can be performed using CH50 and AH50 assays. It has been reported that cell-bound levels of processed complement activation products (like RBC bound C4d) are more sensitive for detecting disease activity in SLE compared to other biomarkers like anti-dsDNA, C3 and C4.[28,29]

Overview of therapeutic strategies

Complement plays a significant role in autoimmune diseases in the form of tissue damage; hence targeting them could be a potential therapeutic regime in these disorders. It has been reported that soluble CR1 inhibits the activation of C3 convertase in dense deposit disease. Giving anti-complement therapy to lupus patients can be deleterious as it can cause infections, autoimmunity, and immune dysregulation. However, supplementing the missing

complements in genetic complement defects can be a better option. For restoring complement activity, plasma infusions supplement complement components but it lasts only for two weeks meriting frequent transfusions. Since C1q is secreted by monocytes in bone marrow, hematopoietic stem cell transplantation (HSCT) is an appropriate option to treat C1q deficiency.[30] Recently a cyclic-pegylated peptide inhibitor of C3, APL-2 is under phase 2 trial in patients with lupus nephritis and other glomerulopathies. Multiple therapeutic agents targeting complement components are under clinical trials.[31] Anti-C5 monoclonal antibodies, eculizumab and ravulizumab, have already been utilized successfully for the treatment of patients with atypical hemolytic uremic syndrome (aHUS).[32] Besides, a subset of patients with paroxysmal nocturnal hemoglobinuria (PNH) also responds to anti-C5 therapy.[33]

Conclusion

It is prudent to consider complement deficiency as one of the underlying etiopathogenetic mechanisms in pediatric autoimmune diseases. Patients with suggestive clinical features must be evaluated comprehensively. Early diagnosis and treatment, facilitated by a high index of suspicion, are crucial for ensuring optimal outcomes in these patients.

Points to Remember

- ***Complement plays a key role in pathogenesis of autoimmune and inflammatory diseases.***
- ***Monogenic lupus can be due to defects in components of classical pathway.***
- ***Complement deficiency results in a predisposition to infections primarily from encapsulated bacteria.***
- ***Defective regulation of complement system can result in atypical hemolytic uremic syndrome or paroxysmal nocturnal hemoglobinuria.***
- ***Excessive alternative pathway activation can cause lupus nephritis or antiphospholipid antibody syndrome.***

References

1. Kaufmann SH. Immunology's foundation: the 100-year anniversary of the Nobel Prize to Paul Ehrlich and Elie Metchnikoff. Nat Immunol 2008; 9:705-712.
2. Varela JC, Tomlinson S. Complement: an overview for the clinician. Hematol Oncol Clin North Am 2015; 29: 409-427.
3. Lintner KE, Wu YL, Yang Y, Spencer CH, Hauptmann G, Hebert LA, et al. Early Components of the Complement Classical Activation Pathway in Human Systemic Autoimmune Diseases. Front Immunol 2016; 15:36.
4. Ballanti E, Perricone C, Greco E, Ballanti M, Muzio GD, Chimenti MS,et al. Complement and autoimmunity. Immunol Res 2013; 56:477-91.
5. Vignesh P, Rawat A, Sharma M, Singh S. Complement in autoimmune diseases. ClinChimActa 2017; 465:123-130.
6. Sharma M, Vignesh P, Tiewsoh K, Rawat A. Revisiting the complement system in systemic lupus erythematosus. Expert Rev Clin Immunol 2020; 16:397-408.
7. Lo MS. Insights Gained From the Study of Pediatric Systemic Lupus Erythematosus. Front Immunol 2018; 9:1278.
8. Bryan AR, Wu EY. Complement deficiencies in systemic lupus erythematosus. Curr Allergy Asthma Rep 2014; 14:448.
9. Macedo AC, Isaac L. Systemic Lupus Erythematosus and Deficiencies of Early Components of the Complement Classical Pathway. Front Immunol 2016; 7:55.
10. Picard C, Lega JC, Ranchin B, Cochat P, Cabrera N, Fabien N, et al. Anti-C1q autoantibodies as markers of renal involvement in childhood-onset systemic lupus erythematosus. Pediatr Nephrol 2017; 32:1537-1545.
11. Ling GS, Crawford G, Buang N, Bartok I, Tian K, Thielens NM, et al. C1q restrains autoimmunity and viral infection by regulating $CD8^+$ T cell metabolism. Science 2018; 360:558-563.
12. Schejbel L, Skattum L, Hagelberg S, Ahlin A, Schiller B, Berg S, et al. Molecular basis of hereditary C1q deficiency - revisited: identification of several novel disease-causing mutations, Genes Immunol 2011; 12:626-634.
13. Truedsson L. Classical pathway deficiencies - A short analytical review. Mol Immunol 2015; 68:14-19.
14. Macedo AC, Isaac L. Systemic lupus erythematosus and deficiencies of early components of the complement classical pathway. Front Immunol 2016; 7:55.
15. Ram S, Lewis LA, Rice PA. Infections of people with complement deficiencies and patients who have undergone splenectomy. Clin Microbiol Rev 2010; 23:740-780.
16. KislaEkinci RM, Altun I, Bisgin A, Atmis B, Altintas DU, Balcý S. Recurrent macroscopic hematuria in a pediatric patient: is it early to diagnose as having type I hereditary C2 deficiency? CEN Case Rep 2020; 9:344-346.
17. Miller EC, Atkinson JP. Overcoming C2 deficiency. Clin Immunol 2012; 144:269-271.
18. Bathia JN, Pal P, Roy M, Guha S. Hereditary Homozygous C3 Deficiency. Indian J Pediatr 2017; 84:643-644.
19. Wu YL, Hauptmann G, Viguier M, Yu CY. Molecular basis of complete complement C4 deficiency in two North-African families with systemic lupus erythematosus. Genes Immun 2009; 10:433-445.
20. Li N, Zhang J, Liao D, Yang L, Wang Y, Hou S. Association between C4, C4A, and C4B copy number variations and susceptibility to autoimmune diseases: a meta-analysis. Sci Rep 2017; 16:42628.

21. Afzali P, Isaeian A, Sadeghi P, Moazzami B, Parvaneh N, Robatjazi M, et al. Complement deficiency in pediatric-onset systemic lupus erythematosus. J Lab Physicians 2018; 10:232-236.
22. Würzner R. Deficiencies of the complement MAC II gene cluster (C6, C7, C9): is subtotal C6 deficiency of particular evolutionary benefit? Clin Exp Immunol 2003; 133: 156-159.
23. Hoare S, Shazali OE, Clark JE, Fay A, Cant AJ. Investigation for complement deficiencey following meningococcal disease. Arch Dis Child 2002; 86:215-217.
24. Mayilyan KR. Complement genetics, deficienceis, and disease associations. Protein Cell 2012; 3:487-496.
25. Hodeib S, Herberg JA, Levin M, Sancho-Shimizu V. Human genetics of meningococcal infections. Hum Genet 2020; 139:961-980.
26. Lisa A, Ram S. Complement interactions with the pathogenic Neisseriae: clinincal features, deficiency states, and evasion mecahnisms. FEBS Letters 2020; 2670-2694.
27. Kavanagh D, Goodship T. Atypical hemolytic uremic syndrome, genetic basis, and clinical manifestations. Hematology 2011; 2011:15-20.
28. Nilsson B, Ekdahl KN. Complement diagnostics: concepts, indications, and practical guidelines. Clin Dev Immunol 2012; 2012:962702.
29. Prohászka Z, Nilsson B, Frazer-Abel A, Kirschfink M. Complement analysis 2016: Clinical indications, laboratory diagnostics and quality control. Immunobiology 2016; 221:1247-1258.
30. Thurman JM, Yapa R. Complement Therapeutics in Autoimmune Disease. Front Immunol 2019; 10:672.
31. Galindo-Izquierdo M, Pablos Alvarez JL. Complement as a Therapeutic Target in Systemic Autoimmune Diseases. Cells 2021; 10:148.
32. Pugh D, O'Sullivan ED, Duthie FA, Masson P, Kavanagh D. Interventions for atypical haemolyticuraemic syndrome. Cochrane Database Syst Rev 2021; 3(3): CD012862.
33. Risitano AM, Marotta S, Ricci P, Marano L, Frieri C, Cacace F, et al. Anti-complement Treatment for Paroxysmal Nocturnal Hemoglobinuria: Time for Proximal Complement Inhibition? A Position Paper from the SAAWP of the EBMT. Front Immunol 2019; 10:1157.

CLIPPINGS

Screen time behavior and Caffeine intake in children.

Screen time (ST) behaviors, for example, television (TV) watching and computer use, among youth are associated with unhealthy eating, and these patterns track over time. A positive association between ST and TV watching with consumption of caffeinated foods and beverages has been described in national samples of children in a few countries. The association of ST behaviors with caffeine intake has not been previously reported. Study conducted to see whether ST behaviors were associated with caffeine intake on a given day (percentage of consumers and amount consumed).

Data on 3421 children (ages 6-11 years) from the cross-sectional National Health and Nutrition Examination Survey 2007-2012 were used. Time spent on TV watching and computer use was determined using questionnaires. Dietary intake was assessed using a 24-hour recall by trained interviewers. Caffeine intake (mg) was estimated by using updated food and nutrient databases. Caffeine consumption was examined in relation to time spent (≥2vs<2hours/day) on ST behaviors.

Children who watched TV ≥2hours/day had significantly higher (~45%more) caffeine intake. Total ST or computer use was not associated with caffeine consumption in school-aged children.

TV watching was positively associated with caffeine intake in school-aged children, suggesting the need for continued monitoring of ST and caffeine intake behaviors in children and adolescents as well as examining the correlates of these behaviors to inform nutrition and health policies.

Ahluwalia N, Frenk SM, Quan SF. Screen time behaviours and caffeine intake in US children: Findings from the cross-sectional National Health and Nutrition Examination Survey (NHANES). BMJ Paediatr Open 2018; 2(1):e000258. Published online 2018 Jun 30. doi:10.1136/bmjpo-2018-000258. PMCID: PMC6045721.PMID: 30019017.

IMMUNOLOGY

SEVERE COMBINED IMMUNE DEFICIENCY

***Ankita Singh**
****Kanika Arora**
*****Pandiarajan Vignesh**

Abstract: *Severe combined immune deficiency is a disorder characterized by defective production or function of lymphocytes resulting in early-onset severe infections in infants. It is a medical emergency and needs to be recognized early for optimal treatment outcomes. Opportunistic infections are the hallmark clinical manifestation and presence of lymphopenia in complete blood counts is a vital clue for diagnosis. Diagnosis can be confirmed by lymphocyte subset analysis with flow cytometry. Hematopoietic stem cell transplantation is the treatment of choice. Establishment of genetic diagnosis is needed for counselling of the affected families.*

Keywords: *Severe combined immune deficiency, Infections, Pneumonia, BCG, Flow cytometry.*

Severe combined immunodeficiency (SCID) is one of the commonest and earliest recognized immunodeficiency. Glanzmann and Riniker reported the first case of SCID in the year 1950 and the first molecular cause [adenosine deaminase (ADA) deficiency] of SCID was recognized in 1972.[1,2] Since then, around 18 genetic defects have been recognized to cause SCID.[3] With the advent of better diagnostic facilities and greater awareness, more cases are being recognized. Newborn screening (NBS) has ushered an era of early diagnosis and management, especially in countries like the United States of America. However, diagnosis is still delayed in developing countries where opportunistic infections in infants with SCID remain the significant cause of mortality. In this review, we aim to highlight points that can aid clinicians in early recognition and provide an algorithmic approach to diagnosis of SCID.

* Senior Resident (DM)
** PhD fellow,
Pediatric Clinical Immunology and Rheumatology
*** Assistant Professor - Pediatrics,
Allergy Immunology Unit, Department of Pediatrics,
Advanced Pediatrics Centre,
Postgraduate Institute of Medical Education
and Research, Chandigarh.
email: vigimmc@gmail.com

Classification and genetics

Various subtypes of SCID can be differentiated by mode of inheritance (X- linked inheritance or autosomal recessive), the immunological phenotype (based on T, B and NK cells) and underlying genetic defects (Fig.1). Typical SCIDs can be classified into following groups based on mechanisms that are affected.

Apoptosis of haematopoietic progenitor cells: Mutations in ADA result in accumulation of adenosine and deoxyadenosine, which are transformed into deoxy- ATP thus impairing the synthesis of other deoxynucleotides. Progenitors of all the three main lymphocyte subsets (T cells, B cells and NK cells) are particularly vulnerable and perish from apoptosis.

Defective cytokine-dependent signalling: Seen in mutations in IL2RG, JAK3 and IL7RA genes.

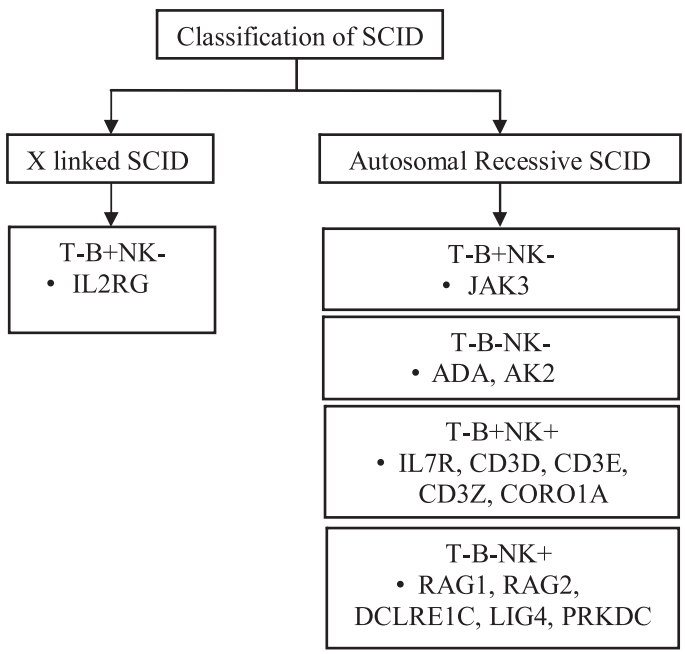


Fig.1. Classification of severe combined immune deficiency

Defective generation of antigen-specific T cell and B cell receptors: Seen in mutations in recombination-activating genes (RAG1 and RAG2), DNA cross-link repair 1C (DCLRE1C), protein kinase DNA-activated catalytic subunit (PRKDC), ligase 4 (LIG4) and Non-homologous end-joining factor 1 (NHEJ1) genes.

Atypical SCID (Leaky SCID): Atypical clinical and immunological presentations of SCIDs ('leaky' SCIDs) occur due to hypomorphic mutations of typical SCID genes resulting in residual protein expression and function.

Epidemiology

Before the establishment of NBS programs, incidence estimated from retrospective studies was around 1 in 100,000 births.[4,5] However, NBS has revealed incidence to be higher up to 1 per 58,000 live births in the USA.[6] It is even higher in countries like Iran (1 per 3000 live births) owning to high consanguinity in population.[7] Similar study from Saudi Arabia revealed incidence 1/2,906 live births after advent of NBS.[8] In Taiwan, first NBS program detected incidence 1 in 53,196 live births.[9] Recently, a large multi-centric study from India described clinical and immunological profile of 254 patients with SCID.[10] This study also highlighted the fact that increasing number of cases are being recognized in India due to better awareness among clinicians and advanced diagnostic facilities. However, most of the cases still go either undiagnosed or are diagnosed late due to absence of NBS. Also limited availability of pediatric transplant facilities contributes to high mortality in these patients.

X-linked SCID [interleukin-2 receptor gamma (IL2RG) mutation] is the most common reported form of SCID. However, genotype incidence and distribution vary based on population structure and consanguinity.[11] Autosomal recessive forms are high in regions with high rates of consanguinity and intra-community marriages.[7] In India, autosomal recessive forms of SCID are commoner than X-linked SCID.

Clinical presentation

SCID is caused by defect in cellular immunity. So in majority of patients, onset of symptoms is in early infancy. However, hypomorphic forms can also present in older patients. Infection profile involves all classes of micro-organisms including bacteria, fungi and viruses (Fig.2). SCID caused by adenosine deaminase (ADA) deficiency show relatively earlier onset of symptoms due to severe lymphopenia. Due to recurrent and severe infections from early infancy, these children also have severe growth failure.

1. Infection profile: Opportunistic infections are the usual presenting manifestation in SCID. As the cellular immunity is impaired, these children can present with any type of viral, bacterial, or fungal infections. Bacterial infections leading to pneumonia, otitis media, septicemia and meningitis are common in these children. Gram negative bacterial infections such as Klebsiella sp., Salmonella sp., and others are also commonly encountered. Children with SCID are often treated as 'late-onset neonatal sepsis'. Presence of lymphopenia in any infant with suspected sepsis warrants evaluation for SCID (Fig.3).

In countries where BCG vaccine is administered at birth, disseminated BCGiosis can be seen in children with SCID and it can be a presenting manifestation in few of them.[10] Disseminated BCGosis manifests as pneumonia, hepatosplenic abscess, osteomyelitis and bone marrow

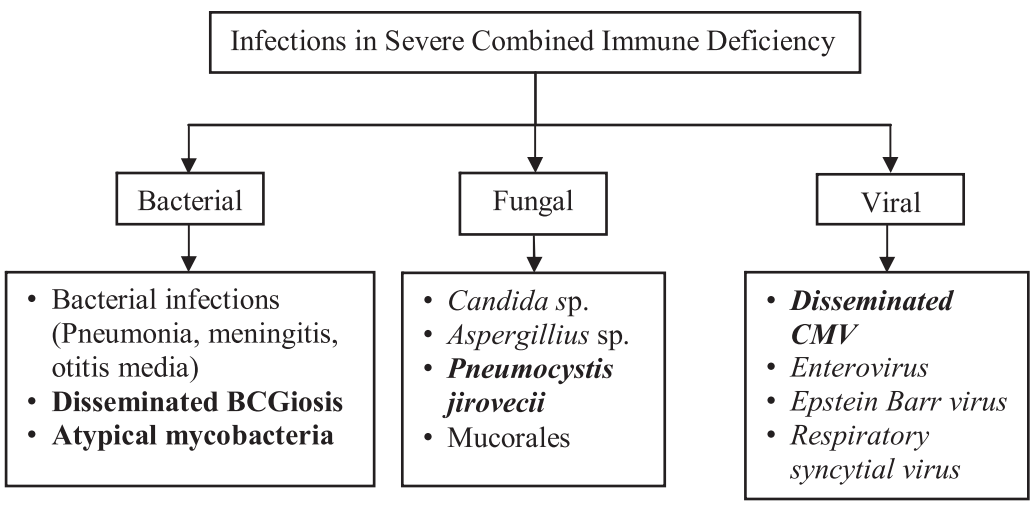


Fig.2. Infection profile in severe combined immune deficiency

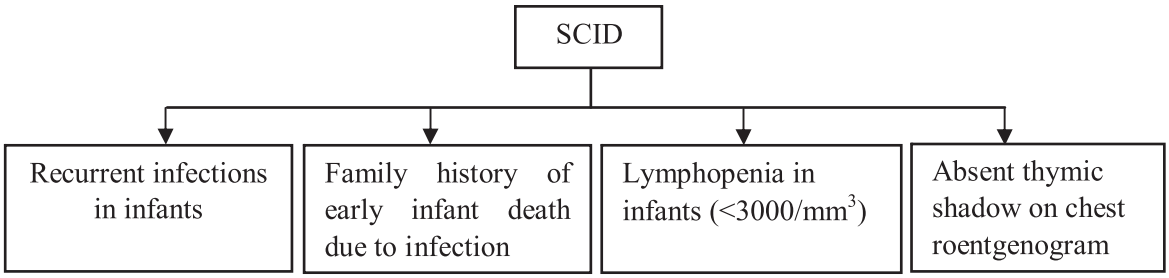


Fig.3. Red flag signs to suspect severe combined immune deficiency

involvement.[12] In any infant with localized BCG-site reaction (such as erythema, ulceration, or pus discharge) who develop hepatosplenomegaly or pneumonia, SCID must be ruled out. Some infants with SCID may not develop scar formation after the vaccination and this can be an important clinical clue.

Viral infections form an important and difficult part of clinical spectrum. Disseminated infections with cytomegalovirus (CMV) and adenovirus contribute to high mortality. Fundus evidence of CMV retinitis is a good bedside clue for disseminated CMV infection and aids in timely initiation of intravenous ganciclovir therapy. Infants with SCID who have disseminated CMV as a presenting manifestation are often misdiagnosed as 'congenital CMV infection'. Respiratory syncytial virus and parainfluenza virus also result in severe pneumonia and respiratory failure in these children. Lymphoproliferation or hematological malignancy due to uncontrolled Epstein-Barr virus (EBV) infection can also be seen in some children.[13] EBV infection also becomes a concern post-transplant in these children.[14]

Fungal infections also contribute to high morbidity and mortality in SCID. Recurrent oral thrush is commonly seen in infants with SCID. Pneumonia due to Pneumocystis jirovecii is frequently seen in children with SCID. Invasive fungal infections with Candida sp. and Aspergillus sp. are common. After human immunodeficiency virus (HIV) infection, SCID is the most common immunodeficiency in which *P.jirovecii* pneumonia is seen. In endemic regions, infections with Penicillium marneffei and Histoplasma capsulatum can also lead to problems.[15] Mucormycosis has also been detected in autopsy studiesin SCID.[16] Chronic diarrhea due to Cryptosporidium parvum, Isospora sp., and Microsporidium sp. can also be seen in children with SCID.

2. Non-infectious complications: Some children with SCID, especially the T-B-NK+ forms of SCID (RAG1, RAG2, DCLRE1C defects) can develop Omenn syndrome characterized by generalized erythematous, exfoliative rash, hepatomegaly, eosinophilia and elevated transaminases. Clinical presentation of Omenn syndrome can sometimes be confused with Langerhans cell histiocytosis or congenital ichthyosis. Presence of generalized erythroderma, loss of scalp hair and eyebrows and presence of eosinophilia are characteristically seen in Omenn syndrome.[17]

Maternal engraftment due to transplacentally derived maternal T cells can lead to clinical manifestations similar to Omenn syndrome.

RAG mutations can be associated with granulomas in skin, mucous membranes and internal organs.[18]

As ADA is ubiquitously expressed, its deficiency can also impair the function of other tissues, including bone (leading to costochondral dysplasia), liver, lung, thymic epithelium and brain tissues. ADA-deficient SCID is associated with skeletal abnormalities, cognitive and behavior abnormalities and pulmonary alveolar proteinosis. Characteristic chest X-ray findings of ADA-deficient SCID include costochondral beading, scapular spur and squaring of lower border of scapula. Microcephaly can be seen in certain subtypes of SCID such as Artemis defects, DNA PK deficiency, Ligase IV deficiency and Cernunnos deficiency. Children with these defects can also develop early and late complications following hematopoietic cell transplantation, growth retardation, endocrinologic deficiencies and dental abnormalities. Bone marrow failure can be seen with Ligase IV deficiency and Cernunnos deficiency. Reticular dysgenesis is associated with sensorineural deafness and agranulocytosis.

Laboratory findings

Lymphopenia is the hallmark that often gives first clue for diagnosis of SCID in infants.[18] However, lymphocyte count can be normal or high in children with Omenn syndrome or maternal T-cell engraftment. Apart from lymphopenia, complete blood counts can show anemia and thrombocytopenia due to presence of opportunistic infections. Inflammatory parameters such as erythrocyte sedimentation rate and C-reactive protein are also elevated due to infections.

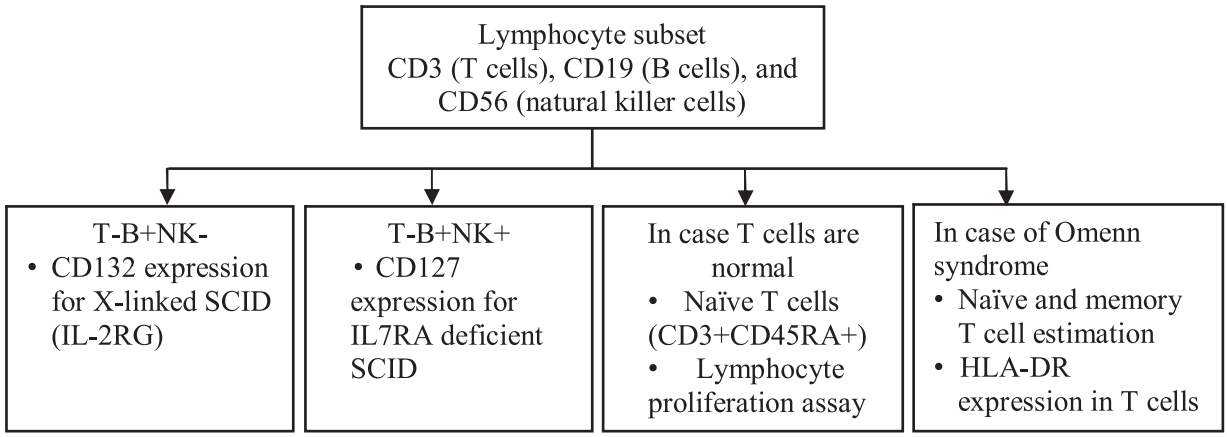


Fig.4. Flowcytometry based investigations in severe combined immune deficiency

Panhypogammaglobulinemia is an important laboratory feature. However, IgG can be normal until 6 months of age due to placental transfer of maternal IgG. Absent thymus, detected by chest roentgenogram and ultrasonography, is another important finding. Chest radiograph can also show features of costochondral dysplasia in cases of ADA-deficient SCID.

Flowcytometry can aid in classifying patients based on lymphocyte subset assay (Fig.4). It can also help in identification of Omenn phenotype. Sub-categories of SCID such as Interleukin 2 Receptor Subunit Gamma(IL2RG) deficient or interleukin 7 (IL-7) receptor alpha (IL7RA) deficient SCID can be identified with help of surface expression of CD132.

Genetic analysis is needed for antenatal diagnosis and counselling. Also, pretransplant genetic diagnosis is required in patients with probability of radiosensitive SCID as they are more sensitive to agents used in conditioning during transplant.[19]

Management

1. Management of infections and supportive therapy: As most infants already acquire opportunistic infections before diagnosis is reached, an important component of their management involves treating these infections before patients can become fit to undergo Human Stem Cell Transplant (HSCT). Antigen-based tests (e.g. polymerase chain reaction) or demonstration of micro-organisms by special stains or culture are needed for microbiological confirmation of infection. As these children do not have functional antibodies, serology-based tests are not reliable in children with SCID to rule in or rule out infections.

Immunoglobulin replacement therapy also forms an important part that has to be started once diagnosis is made as most patients have either agammaglobulinemia or hypogammaglobulinemia. These patients also suffer from severe malnutrition and require nutritional rehabilitation. Another important component of management involves transfusing irradiated blood products whenever blood transfusion is required. This is needed to prevent transfusion-acquired CMV infection.

Empirical cover for P. jirovecii with intravenous cotrimoxazole is needed for children with pneumonia in addition to the routine anti-microbials. Work-up for CMV, other respiratory viruses, and fungal infections are needed in these children in addition to the work-up for bacterial etiology.

In countries like India where universal BCG vaccination at birth is practised, prophylactic therapy with isoniazid and rifampicin is required if there is no evidence of disseminated BCGiosis at presentation. The prophylactic therapy is usually continued until successful engraftment post-HSCT. Children with SCID who have evidence of disseminated BCGiosis must be preferably treated with 4 drug anti-tubercular therapy (isoniazid, rifampicin, ethambutol and levofloxacin). Pyrazinamide is generally not added in treatment of BCGiosis as BCG has intrinsic resistance to this drug.

Probiotics must not be used in the management of diarrhea in these children as the organisms used can lead to septicemia.

If the child is very sick, EDTA blood samples must be immediately stored for genetic analysis. Confirmation

of genetic diagnosis is preferably needed for antenatal diagnosis in subsequent pregnancies and parents must be counselled accordingly.

2. Definitive therapy: HSCT remains cornerstone of definitive therapy in SCID.[20] Children with SCID must be referred early to a centre where facilities for HSCT are available. Timely HSCT is essential for good outcomes.

   Other treatment options available for some variants include enzyme replacement therapy (ERT) and gene therapy.

Gene therapy is the emerging therapeutic option in patients who do not have HLA matched donors. It has shown good results in ADA and IL2RG SCID and is being investigated in other forms of SCID.[21, 22]

Vaccination

All live vaccines are contraindicated in SCID and inactivated vaccines are not useful due to lack of antibody production.[23]

Newborn screening

Several countries have started newborn screening (NBS) programs that aid in detecting SCID in newborns before they develop any opportunistic infections. This provides an important window to proceed with early transplantation before child has infections and thus, significantly improves survival rates. NBS for SCID involves assaying T-cell receptor excision circles (TREC) in dried blood spots, which is a surrogate marker of T-cell production.[24]

NBS was first started in USA and they found that incidence of SCID after introduction of NBS was significantly more than that considered previously. Subsequently several countries in Europe and Asia have also started incorporating it in their programs.[25]

Points to Remember

- ***Severe Combined Immune Deficiency (SCID) is a severe form of primary immunodeficiency disorder characterised by defective lymphocyte production or function.***
- ***Clinical manifestations in SCID usually start from early infancy. These include opportunistic infections which are recurrent and severe.***
- ***In countries where universal BCG vaccination at birth is practiced, disseminated BCGosis remains a major concern in children with SCID.***
- ***Presence of lymphopenia (absolute lymphocyte counts in infants <3000/mm^3) is an important laboratory clue.***
- ***Flow cytometry enumeration of lymphocyte subsets helps in diagnosing and categorising subtype of SCID.***
- ***Hematopoietic stem cell transplantation (HSCT) is the definitive mode of therapy for children with SCID.***
- ***Early identification and timely HSCT results in successful outcomes in SCID.***

References

1. Glanzmann E, Riniker P. Essential lymphocytophthisis; new clinical aspect of infant pathology. Ann Pediatr 1950; 175(1-2):1-32.
2. Giblett ER, Anderson JE, Cohen F, Pollara B, Meuwissen HJ. Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. Lancet 1972; 2(7786):1067-1069.
3. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. J Clin Immunol 2020; 40(1):66-81.
4. Ryser O, Morell A, Hitzig WH. Primary immunodeficiencies in Switzerland: first report of the national registry in adults and children. J Clin Immunol 1988; 8(6):479-485.
5. Stephan JL, Vlekova V, Le Deist F, Blanche S, Donadieu J, De Saint-Basile G, et al. Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 patients. J Pediatr 1993; 123(4):564-572.
6. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA 2014; 312(7): 729-738.
7. Shahbazi Z, Yazdani R, Shahkarami S, Shahbazi S, Hamid M, Sadeghi-Shabestari M, et al. Genetic mutations and immunological features of severe combined immunodeficiency patients in Iran. Immunol Lett 2019; 216:70-78.
8. Al-Mousa H, Al-Dakheel G, Jabr A, Elbadaoui F, Abouelhoda M, Baig M, et al. High Incidence of Severe Combined Immunodeficiency Disease in Saudi Arabia Detected Through Combined T Cell Receptor Excision Circle and Next Generation Sequencing of Newborn Dried Blood Spots. Front Immunol 2018; 9:782. doi: 10.3389/fimmu.2018.00782. PMID: 29713328; PMCID: PMC5911483.
9. Chien YH, Chiang SC, Chang KL, Yu HH, Lee WI, Tsai LP, et al. Incidence of severe combined

immunodeficiency through newborn screening in a Chinese population. J Formos Med Assoc 2015; 114(1):12-16.

10. Vignesh P, Rawat A, Kumrah R, Singh A, Gummadi A, Sharma M, et al. Clinical, Immunological, and Molecular Features of Severe Combined Immune Deficiency: A Multi-Institutional Experience From India. Front Immunol 2021; 11:619146. DOI=10.3389/fimmu. 2020. 619146
11. Fischer A. Severe combined immunodeficiencies (SCID). Clin Exp Immunol 2000; 122(2):143-149.
12. Norouzi S, Aghamohammadi A, Mamishi S, Rosenzweig SD, Rezaei N. Bacillus Calmette-Guérin (BCG) complications associated with primary immunodeficiency diseases. J Infect 2012; 64(6):543-554.
13. Meggetto F, Muller C, Henry S, Selves J, MariamÝB, Brousset P, et al. Epstein-Barr Virus (EBV)-Associated Lymphoproliferations in Severe Combined Immunodeficient Mice Transplanted With Hodgkin Disease Lymph Nodes: Implication of EBV-Positive Bystander B Lymphocytes Rather Than EBV-Infected Reed-Sternberg Cells. Blood 1996; 87(6):2435-2442.
14. Monforte-Muñoz H, Kapoor N, Saavedra JA. Epstein-Barr virus-associated leiomyomatosis and posttransplant lymphoproliferative disorder in a child with severe combined immunodeficiency: case report and review of the literature. Pediatr Dev Pathol 2003; 6(5):449-457.
15. Lee PPW, Lau YL. Endemic infections in Southeast Asia provide new insights to the phenotypic spectrum of primary immunodeficiency disorders. Asian Pac J Allergy Immunol 2013; 31(3):217-226.
16. Gupta K, Rawat A, Agrawal P, Jindal A, Nada R, Saikia B, et al. Infectious and non-infectious complications in primary immunodeficiency disorders: an autopsy study from North India. J Clin Pathol 2018; 71(5):425-435.
17. Villa A, Santagata S, Bozzi F, Giliani S, Frattini A, Imberti L, et al. Partial V(D)J recombination activity leads to Omenn syndrome. Cell 1998; 93(5):885-896.
18. Aluri J, Gupta MR, Dalvi A, Mhatre S, Kulkarni M, Desai M, et al. Lymphopenia and Severe Combined Immunodeficiency (SCID) - Think Before You Ink. Indian J Pediatr 2019; 86(7):584-589.
19. Dvorak CC, Cowan MJ. Radiosensitive severe combined immunodeficiency disease. Immunol Allergy Clin North Am 2010; 30(1):125-142.
20. Grunebaum E, Mazzolari E, Porta F, Dallera D, Atkinson A, Reid B, et al. Bone marrow transplantation for severe combined immune deficiency. JAMA 2006; 295(5):508-518.
21. Carbonaro DA, Zhang L, Jin X, Montiel-Equihua C, Geiger S, Carmo M, et al. Preclinical demonstration of lentiviral vector-mediated correction of immunological and metabolic abnormalities in models of adenosine deaminase deficiency. Mol Ther 2014; 22(3):607-622.
22. Touzot F, Moshous D, Creidy R, Neven B, Frange P, Cros G, et al. Faster T-cell development following gene therapy compared with haploidentical HSCT in the treatment of SCID-X1. Blood 2015; 125(23):3563-3569.
23. Sobh A, Bonilla FA. Vaccination in Primary Immunodeficiency Disorders. J Allergy Clin Immunol Pract 2016; 4(6):1066-1075.
24. Puck JM. Newborn Screening for Severe Combined Immunodeficiency and T-cell Lymphopenia. Immunol Rev 2019; 287(1):241-252.
25. Gerstel-Thompson JL, Wilkey JF, Baptiste JC, Navas JS, Pai S-Y, Pass KA, et al. High-throughput multiplexed T-cell-receptor excision circle quantitative PCR assay with internal controls for detection of severe combined immunodeficiency in population-based newborn screening. Clin Chem 2010; 56(9):1466-1474.

CLIPPINGS

Serum Amyloid A in Early-Onset Sepsis in Premature Infants.

Preterm newborns with suspected early-onset neonatal sepsis were evaluated at admission and at the 24th and 48th hours after admission. The serum amyloid A values of the patients with sepsis and a control group compared, and the blood cultures evaluated.

A total of 319 premature newborns 150 in the sepsis group and 169 in the control group, the birth weight ranged between 590 g and 3000 g and gestational age was 24-36 weeks. The serum amyloid A values at admission were significantly higher in neonates with sepsis.

Serum amyloid A is a reliable diagnostic marker for the early onset of neonatal sepsis.

Dorum BA, Özkan H, Çakir SC, Köksal N, Gözal Z, Çelebi S, et al. The Diagnostic Value of Serum Amyloid A in Early-Onset Neonatal Sepsis in Premature Infants. HK J Paediatr (New Series) 2021; 26:8-13.

IMMUNOLOGY

DISORDERS OF IMMUNE REGULATION

***Sathish Kumar**
****Anu Punnen**

Abstract: *Inborn errors of immunity are genetic disorders with broad clinical manifestations, ranging from increased susceptibility to infections to significant immune dysregulation. As per 2019 Update of the International Union of Immunological Societies expert committee's classification, there are now 430 single-gene inborn errors of immunity. Primary immune regulatory disorders are a growing subset of diseases referred to as inborn errors of immunity. Unlike classical primary immune deficiency disorders that typically present with severe, recurrent, or unusual infections, the clinical manifestations of primary immune regulatory disorders are dominated by immune-mediated diseases (autoimmunity, autoinflammation/ hyperinflammation, lymphoproliferation, malignancy, and severe atopy). In this article we will discuss in detail about disorders of immune regulation with phenotypical presentation and associated genetic defects.*

Keywords: *Immune deficiency, Inborn errors of immunity, Primary immune regulatory disorders, Autoimmunity.*

Primary immunodeficiency diseases (PIDs) or inborn errors of immunity (IEI) are inherited disorders that impair the immune response, leading to increased risk of infections, immune dysregulation, autoimmune phenomena, inflammation and malignancy. IEI now comprise 406 distinct disorders with 430different gene defects listed in the 2019 International Union of Immunological Societies (IUIS) classification.[1] There are 10 main groups of phenotypic algorithm for classification. Group 6 is classified as diseases of immune dysregulation. Primary immune regulatory disorders (PIRD) arise from a breakdown in immune regulation resulting in clinical phenotypes such as autoimmunity, autoinflammation/ hyperinflammation, lymphoproliferation, malignancy and severe atopy.[2]

A total of 430 gene defects associated with inborn errors of immunity were reported in the International Union of Immunological Societies of which 129 are considered a primary immune regulatory disorder (Fig.1).

This article describes the clinical and laboratory features of PIRDs, diagnosis and treatment strategies aimed to control the disease burden in affected children.

Three different clinical/immunophenotypic categories are frequently encountered: 1. autoimmune lymphoproliferative syndrome (ALPS) and ALPS-like syndromes, 2. immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) and IPEX-like disorders and 3. common variable immunodeficiency (CVID), CVID-like and late-onset combined immune deficiency (late-onset CID) disorders as depicted in Table I.

Clinical presentation of primary immune regulatory disorders

The spectrum of primary immune regulatory disorders can be broadly classified in the following patterns: [3]

1. ALPS/ALPS-like: Autoimmune cytopenias (AIC) and lymphoproliferation are frequent

2. IPEX/IPEX-like: Enteropathy and endocrinopathy with or without AIC and other autoimmune manifestations are frequent

3. CVID/CVID-like/late-onset CID: Chronic or recurrent infections, AIC, other autoimmunity and lympho-proliferation are frequent.

The overlap between clinical and immunologic phenotypes of primary immune regulatory disorders is illustrated in Fig.2 and Fig.3.

ALPS/ALPS-like disorders

Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of dysregulated lymphocyte apoptosis, which

* Professor of Pediatrics

** Associate Professor,
Pediatric Rheumatology,
Department of Pediatrics,
Christian Medical College,
Vellore.

email: sathishkumar@cmcvellore.ac.in

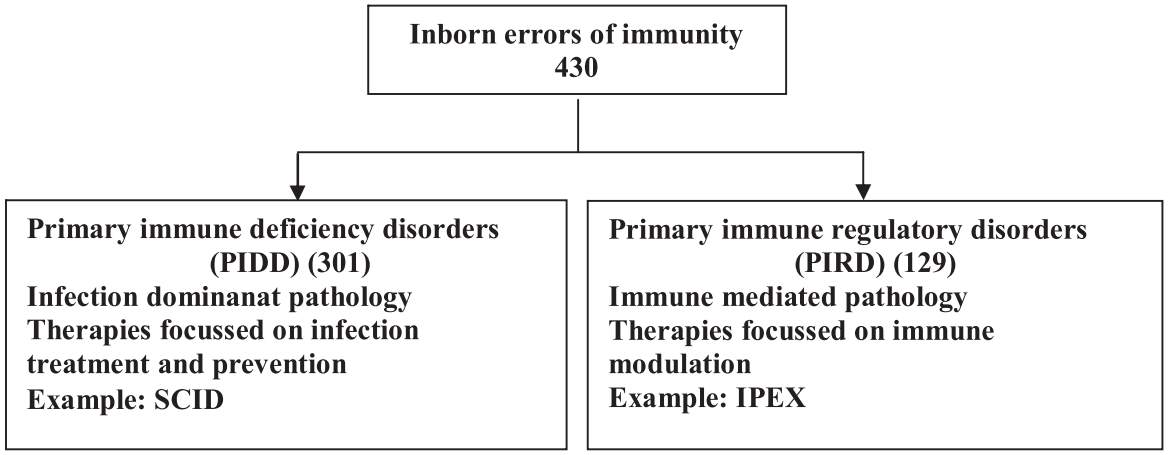


Fig.1. Inborn errors of immunity

is marked by chronic lymphoproliferation and autoimmunity.[4] Clinical features of ALPS include chronic lymphadenopathy or splenomegaly. ALPS most commonly manifests as multi-lineage immune-mediated cytopenias that are often episodic. A negative ALPS workup, lymphocytic infiltration of non-lymphoid organs (e.g., lung, gut, central nervous system) as well as chronic infections (bacterial, viral, fungal) should alert pediatricians to consider the possibility of an ALPS-like disorder or other primary immune regulatory disorders.[5] ALPS is usually associated with highly elevated levels of specific biomarkers including soluble FAS ligand (sFASL), vitamin B12, and interleukins 10 (IL-10) and 18 (IL-18). B12 and sFASL are particularly predictive of a mutation in FAS.

Elevations in CD3+CD4-CD8- double-negative T cells (DNTCs) is required for a diagnosis of ALPS, A lack of DNTC elevation or a mild elevation may suggest an ALPS-like disorder. Defective apoptosis, somatic/germline mutations in FAS/FASLG/CASP10 are also characteristic of ALPS that is caused by defect in FAS or FASLG12.

There is considerable overlap between the immunologic features of ALPS and ALPS-like disorders (e.g., normal or elevated serum IgG).Aberrations in antibody responses (e.g., poor responses) and lymphocyte immunophenotyping (e.g., diminished CD19+CD27+ memory B cells as depicted in Fig.2) may be seen in both ALPS and ALPS-like disorders. Histologic examination of lymphatic tissue revealing the characteristic findings of follicular hyperplasia with a prominent expansion of paracortex with DNTCs should prompt consideration of an ALPS.

Genetic testing should be performed to confirm a diagnosis of ALPS (germline/ somatic mutation in FAS, FASLG, CASP10). Of note, Ras-associated autoimmune leukoproliferative disorder (RALD) is associated with the presence of activating somatic mutations in Ki-ras2 Kirsten rat sarcoma (KRAS) or neuroblastoma RAS viral oncogene homolog (NRAS).[6]

Advances in sequencing have led to the discovery of a variety of other monogenic defects that are associated with an ALPS-like phenotype including autosomal recessive disorders such as loss-of-function mutations in LPS-Responsive-Beige-like Anchor1 (LRBA1) and autosomal dominant disorders such as Cytotoxic T-Lymphocyte Associated Protein 4(CTLA4) haploinsufficiency and gain-of-function mutations in Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Delta (PIK3CD) and Signal transducer and activator of transcription 3 (STAT3) as examples.[7]

Immune dysregulation, polyendocrinopathy, enteropathy (IPEX)/IPEX-like disorders. IPEX is the prototypic disorder resulting from defective Tregs.[8] It is caused by mutations in Forkhead Box P3 (FOXP3), a critical transcription factor required for development of functional Tregs. IPEX patients typically manifest striking features of autoimmunity including enteropathy, endocrinopathies, and dermatitis; however, other non-infectious, autoimmune features are common (e.g., AIC, autoimmune hepatitis). Chronic viral, fungal and bacterial infections are unusual in IPEX and should alert clinicians to consider the possibility of an IPEX-like disorder.

FOXP3 expression may be diminished or absent in patients with IPEX; however, patients with missense mutations in FOXP3 or variants that impact function may have normal FOXP3 expression. Patients with IPEX-like disorders may also have normal or decreased FOXP3 expression.[9] Importantly, IPEX and IPEX-like disorders such as those that involve the perturbation of CTLA4

Table I. Clinical features suggestive of primary immune regulatory disorders

Category	Clinical features	Laboratory features	Histologic features
ALPS	- Lymphadenopathy - Splenomegaly - Autoimmunity - particularly recurrent episodic cytopenias	- Increased CD3+CD4- CD8- TCR α/β DNTC > 2.5 % - Elevated sFASL, vitamin B12, IL- 10 - Normal IgG - Abnormal FAS mediated apoptosis - Germline mutation in FAS, FASLG or CASP10 or somatic mutation in FAS	- Lymph nodes show paracortical expansion with DNTCs
ALPS-like disorders	- Lymphadenopathy - Splenomegaly - Autoimmunity - Chronic viral, fungal, bacterial infections	No DNTC elevation or mild elevation - Normal or mild elevation in sFASL, IL-10 and B12 - Low IgG and IgA or elevations in IgM - Poor antibody responses - Diminished memory B cells CD19+CD27+ - Normal apoptosis - No somatic/germline mutation in FAS, FASLG, CASP10	- No paracortical expansion with DNTCs - Lymphocytic infiltration of nonlymphoid organs (gut, lung or brain)
IPEX	- Enteropathy - Endocrinopathies (type I DM or thyroiditis) - Dermatitis (eczema) - Autoimmunity	- Absent or decreased FOXP3 expression in Tregs - Abnormal Tregs numbers or function - Significantly elevated IgE - Elevated eosinophils - Mutation in FOXP3	GI tract: -Villous atrophy in small bowel - Apoptosis of enterocytes - Lymphocytic infiltration - Eosinophils - Depletion of goblet cells Skin: - Eczematous
IPEX-like disorders	- Enteropathy - Endocrinopathies - Eczema - Autoimmunity - Chronic viral, fungal, bacterial infections	- Normal to mild decrease in FOXP3 expression - Abnormal Treg numbers or function - Normal/mild elevation IgE - No or mild elevations in eosinophils - B-cell numbers low - T-cell numbers low - Absence of FOXP3 mutation	GI tract: - Villous atrophy - Apoptosis of enterocytes - Graft-versus host disease-like - Lymphocytic infiltration - Eosinophils - Depletion of goblet cells Skin: - Spongiotic dermatitis
CVID-like or late-onset CID disorders	- Sinopulmonary infection - Granulomatous inflammation - Enteropathy - Lymphoproliferation - Autoimmunity - Young age of onset	- Low IgG and IgA or elevations in IgM - Poor antibody responses - Abnormal lymphocyte immunophenotyping - Abnormal mitogen responses	GI tract: - Villous atrophy - Lymphocytic infiltration - Eosinophils - Depletion of goblet cells - Granulomatous inflammation (lung, lymph nodes, liver, skin) - Lymphocytic infiltration of non-lymphoid organs (gut, lung or brain)

Category	Clinical features	Laboratory features	Histologic features
Hyperinflammatory disorders (HLH-like)	- Persistent fevers - Pancytopenia - Hepatosplenomegaly - Hepatitis or acute liver failure - Altered mental status / seizures - MAS at a young age	- Elevations in ferritin, in particular soluble IL-2 receptor not as elevated as in typical HLH - Normal or increased perforin and granzyme B expression - Normal CD107a degranulation - Normal SAP expression - Absent or reduced XIAP expression (in XIAP deficiency) - Elevated IL-18 - Mutations in PRF1, UNC13 D, STXBP2, STX11, RAB27A, LYST, AP3B1	- Hemophago-cytosis (bone marrow, liver or CSF) - Increased CD163 staining suggestive of activated macrophages in involved organs
Immune defects associated with lymphohemato-poietic	- Lymphoproliferation - Relapsed disease - Young age of onset - Developmental delay (in DNA-repair defects)	- Poor NK cell degranulation - Poor T-cell function - Low CD4 counts - High senescent CD8 T cells - High EBV plasma load	- Early-onset mature lymphoma - EBV positivity - High stage - GI/CNS involvement

Abbreviations: DNTC - double negative T-cells; PRF1-Perforin; UNC 13 D-Protein unc-13 homolog D; STXBP2-Syntaxin Binding Protein 2, STX11-Syntaxin 11 human gene member of the t SNARE family; RAB27A-Ras-related protein Rab 27A is aprotein encoded by RAB 27A gene; AP3B1-Adapter LYST gene provides protein known as lysosomal trafficking regulator; Related Protein Complex 3 Subunit Beta 1 a protein coding gene.

(Source: Chandrakasan S, Chandra S, Davila Saldana BJ, Torgerson TR, Buchbinder D. Primary immune regulatory disorders for the pediatric hematologist and oncologist: A case-based review. Cancer 2019; 66(5):e27619).

(e.g., CTLA4 haploinsufficiency or LRBA deficiency) may demonstrate abnormal numbers of Tregs. STAT1-GOF mutations are frequently associated with chronic mucocutaneous candidiasis as well as a variety of other fungal, bacterial, viral infections and organ-specific autoimmunity (e.g., thyroid dysfunction). Patients with STAT1-GOF mutations may require additional testing, such as Th17 enumeration, which demonstrates decreased numbers of Th17 cells.[10]

Elevated IgE and eosinophilia are common in IPEX but less common in IPEX-like disorders. Immunoglobulins may be diminished secondary to intestinal loss or to defects in B-cell isotype switching. Moreover, immunoglobulin levels along with B-cell numbers and function may become progressively diminished over time, as is frequently observed in IPEX-like disorders (e.g., CTLA4 haploinsufficiency and LRBA deficiency). Histologic examination of the gastrointestinal tract may demonstrate villous atrophy, lymphocytic infiltration, the presence of eosinophils and apoptosis of enterocytes, which in some cases can mimic graft-versus-host disease-like pathology. Goblet cells may also be depleted, often by an autoimmune process. Similarly, skin biopsies may demonstrate nonspecific features such as spongiotic dermatitis. These findings may also be seen in both IPEX and/or IPEX-like disorders. Different mutations have different inheritance. (e.g. IPEX is X-linked recessive; STAT1-GOF and STAT3-GOF are autosomal dominant; LRBA deficiency is autosomal recessive).

Advances in genetic sequencing have identified an expanding list of monogenic defects that are associated with an IPEX-like phenotype (e.g. CTLA4, LRBA, STAT1-GOF and STAT3-GOF; Fig.2).

Common variable immunodeficiency (CVID), CVID-like and late-onset combined immunodeficiency disorders

CVID It is thought to be due predominantly to defects in B-cell homeostasis (e.g. class switch recombination). Hypogammaglobinemia and recurrent bacterial sinopulmonary infections are the hallmark of CVID; however, patients may manifest autoimmunity such as enteropathy and endocrinopathies.[11] A variety of

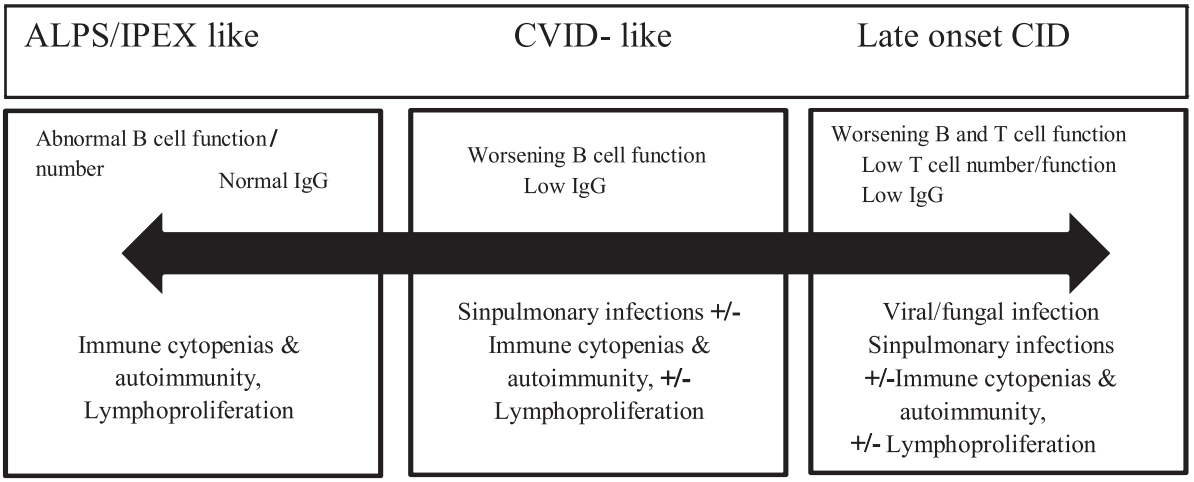


Fig.2. Spectrum of clinical presentation and genetics of primary immune regulatory disorders

Genetic defects associated with PIRD

Biallelic defects - LRBA, RAG1, RAG2, DOCK8, STAT5B, WIP, CASP8, FADD, TPP2

Gain of function - JAK1, STAT1, STAT3, PIK3CD, PI3KR1

Haploinsufficiency - CTLA4, NFKB1, NFKB2, NFAT5, BACH2, PTEN

Dominant Negative - FAS, FASLG, CARD11

X-linked - FOXP3, IKBKG, WAS, MAGT1

Somatic mutations - FAS. KRAS, NRAS

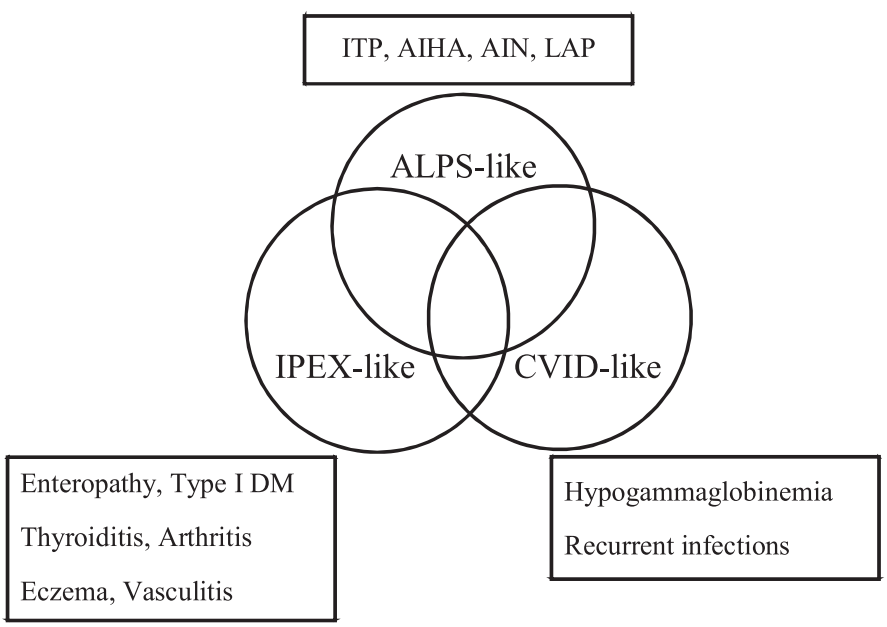


Fig.3. Clinical phenotypes of primary immune regularity disorders. ITP - Immune thrombocytopenic pupura; AIHA - Autoimmune hemolytic anemia; AIN - Autoimmune neutropenia; LAP - Lymphadenopathy and lymphoproliferation; Type I DM - diabetes mellitus

(Source: Chandrakasan S, Chandra S, Davila Saldana BJ, Torgerson TR, Buchbinder D. Primary immune regulatory disorders for the pediatric hematologist and oncologist: A case-based review. Pediatr Blood Cancer 2019; 66(5):e27619).

non-infectious pulmonary features are also frequently observed including lymphocytic interstitial pneumonia, follicular bronchiolitis and granulomatous-lymphocytic interstitial lung disease.

Classic features of CVID include recurrent sinopulmonary infection secondary to hypogammaglobulinemia and poor antibody production. Other non-infectious complications are also seen in both

Table II. Hemophagocytic Lymphohistiocytosis

	Conditions	Gene defect	Clinical features
Hypopigmentation Partial albinism decreased NK and CTL activities (cytotoxicity and/or degranulation). B cells and T cells: Normal	Chediak Higashi Syndrome	LYST	Recurrent infections, fever, HSM, bleeding tendency, progressive neurological dysfunction, giant lysosomes, neutropenia, cytopenias, specific hair shaft anomaly Increased activated T cells
	Griscelli Syndrome type 2	RAB27A	Fever, HSM, cytopenias; Specific hair shaft anomaly
	Hermansky Pudlak syndrome type 2	AP3B1	Infections, pulmonary fibrosis,bleeding, neutropenia; Specific hair shaft anomaly
	Hermansky Pudlak syndrome, type 10	AP3D1	Oculocutaneous albinism, severe neutropenia, recurrent infections, seizures, hearing loss and neurodevelopmental delay
Familial Hemophagocytic Lymphohistiocytosis Syndromes	Perforin deficiency (FHL2)	PRF1	Fever, HSM, cytopenias, normal B cells.
	UNC13D / Munc13-4 deficiency (FHL3).	UNC13D	Increased activated T cells. Decreased to absent NK and CTL activities (cytotoxicity and/or degranulation).
	Syntaxin 11 deficiency (FHL4)	STX11	
	STXBP2 / Munc18-2 deficiency (FHL5) Enteropathy	STXBP2	
	FAAP24 deficiency	FAAP24	EBV driven lymphoproliferative disease. Increased activated T cells. Failure to kill autologous EBVtransformed B cells. Normal NK cell function.
	SLC7A7 deficiency	SLC7A7	Lysinuric protein intolerance, bleeding tendency, alveolar proteinosis, Hyperinflammatory response of macrophages. Normal T cells and NK cell function

Abbreviations: LYST - Lysosomal Trafficking Regulator; AP3B1- Adaptor Related Protein Complex 3 Subunit Beta 1; PRF1- Perforin-1; UNC13D- Unc-13 Homolog D; STX11 - Syntaxin 1; STXBP2 - Syntaxin Binding Protein 2; FAAP24- Fanconi anemia core complex-associated protein 24; SLC7A7 - solute carrier family 7 (amino acid transporter light chain, y+L system)

(Source: Tangye SG, Al-Herz W, Bousfiha A,Chatila T, CunninghamRundles C, Etzioni A, et al. Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 2020; 40:24-64).

Table III. Example of targeted therapies for children with genetically defined primary immune regulatory disorders

Condition	Gene	Targeted therapy
IPEX	FOXP3	Tacrolimus, Cyclosporin Sirolimus
STAT1 GOF	STAT1	Ruxolitinib (JAK 1/2 inhibitor) Sirolimus
STAT3 GOF	STAT3	Tocilizumab (IL-6 receptor blocker) Siltuximab (IL-6 blocker) Ruxolitinib (JAK 1/2 inhibitor)
LRBA deficiency	LRBA	Abatacept, sirolimus Hydroxychloroquine
CTLA4 haploinsufficiency	CTLA4	Sirolimus, abatacept
APDS	PIK3CD PIK3R1	Sirolimus Leniolisib (PI3K inhibitor)
XIAP and NLRC4	BIRC4, NLRC4	IL-18 binding protein
Primary HLH	PRF1, UNC13D, STX11, STXBP2	Emapalumab (IFN- blocking antibody) Ruxolitinib (JAK 1/2 inhibitor)

CVID and CVID-like disorders (e.g. granulomatous inflammation, enteropathy, lymphoproliferation, malignancy and organ specific autoimmunity such as autoimmune hepatitis) (Fig.3).

Hypogammaglobulinemia with poor vaccine titers and documentation of poor antibody response to revaccination are commonly found in both CVID and CVID-like disorders. Lymphocyte immunophenotyping and in vitro proliferation of T cells in response to mitogens is typically normal in CVID and increase in circulating T follicular helper cells. Histologic examination of patients with CVID-like disease with AIC often shows expanded but bizarre arrangement of germinal centers. Granulomatous inflammation involving the lung, lymph nodes, liver and skin may also be present. These findings may be seen in both CVID and/or CVID-like disorders; however, their presence as well as lymphocytic infiltration of other non-lymphoid organs such as gut, lung, or brain should prompt consideration of a CVID-like disorder. Genetic evaluation of CVID-like disorders is required when classic features of CVID including hypogammaglobulinemia and poor antibody responses resulting in recurrent sinopulmonary infection are coupled with immune dysregulation such as inflammatory complications and/or organ-specific autoimmunity such as immune cytopenias. Aberrations in a growing number of genes including Nuclear Factor Kappa B Subunit 1(NFKB1), NFKB2, IKAROS Family Zinc Finger 1 (IKZF), CTLA4, LRBA, STAT1, STAT3 and others have been demonstrated in CVID-like disorders.

Hyperinflammatory disorders (HLH-like)

HLH is a life-threatening disorder characterized by uncontrolled, excessive cytotoxic lymphocyte and macrophage activation. Although HLH typically presents in an infant or young child with persistent fevers, pancytopenia, and hepatosplenomegaly(HSM), it can present with a variety of other clinical features including hepatitis or acute liver failure or altered mental status/ seizures. Elevated ferritin and soluble IL2R are diagnostic biomarkers for HLH. Once the diagnosis of HLH is made, it is important to determine if HLH is primary (underlying genetic basis) or secondary, because primary HLH will eventually need allogeneic HSCT. Clinical phenotype can be divided into children with albinism and with familial HLH. Table II demonstrates HLH phenotype with albinism and familial HLH conditions.

Treatment of HLH

The mainstays of HLH treatment consist of immunosuppressive and chemotherapeutic drugs and biologics that aim to dampen the cytokine storm and eliminate activated T-cell and macrophage populations. A commonly used treatment approach consists of

dexamethasone and etoposide based on the experiences of the Histiocyte Society HLH-2004 studies.[12,13]

Rituximab can be helpful in the treatment of EBV-HLH. Various treatment modalities like IV immunoglobulin, therapeutic plasma exchange, anakinra (recombinant IL-1 receptor antagonist) and/or corticosteroids to stop inflammation while workup is ongoing, although data supporting these practices are lacking.[14]

Allogeneic HCT is indicated in many pediatric patients with genetic HLH if a suitable donor is available, including patients with pathologic variants in PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST and SH2D1A and some patients with XIAP deficiency.

Evaluating and diagnosing primary immune regulatory disorders

Given the wide clinical spectrum of PIRD, evaluation is focused on identifying the predominant clinical features like enteropathy, dermatitis, endocrinopathy and cytopenias. A multidisciplinary team approach including pediatrician, immunologist and medical genetist is essential for gathering data and decide on clinical phenotype based on above tables. The absence of a positive family history does not eliminate a genetic cause as PIRD can be associated with heterozygous defects causing dominant gain-of-function or loss of-function so can arise de novo. As a result, identification of a defect in a PIRD-associated gene should be considered based on its immunologic function. Broad-based genetic testing (e.g., exome sequencing, targeted gene panel) is often the most informative and should be considered early in the evaluation.

Overview of treatments for primary immune regulatory disorders

Ideal therapy for children with PIRD is targeted at the specific genetic defect.[15] Targeted specific immune modulatory medications are available now for children with PIRD, however long term data is lacking. Children with inadequate response and unacceptable side-effects require more definitive therapy such as HCT or gene therapy.

Immunomodulatory therapies

Understanding the specific immune pathway defect is important to treat children with PIRD. Example of targeted therapies for children with genetically defined primary immune regulatory disorders are tabulated in Table III.

Hematopoietic cell transplant

HCT can be a successful treatment option for PIRD patients depending on the genetic defect and other transplant conditions.[16] Better control of the hyperinflammatory process prior to transplant may help decrease the risk for alloreactivity and potentially improve engraftment of donor cells. At present, data are lacking about the best conditioning regimens for patients with PIRD. Overall, HCT can be a potential therapy for PIRD but significant questions remain

Conclusion

Improved understanding of the molecular mechanisms of several newly described PIRDs allowed for the development of targeted therapeutic strategies to treat affected children. In children in whom HSCT is required, targeted treatments have been successful in decreasing pre transplant disease burden. Newer drugs are used as bridge therapy to control immune dysregulation and hyperinflammation in children with PIRD prior to HSCT and improving HSCT outcomes.

Points to Remember

- ***PIRD predominantly have clinical features of autoimmunity, hyperinflammation, lymphoproliferation, malignancy and severe atopy with less dominant features of immunodeficiency and infection.***
- ***Genetic causes of PIRD function in immune pathways that regulate the various types of immune responses.***
- ***The treatment is challenging, as it requires careful balancing of immunosuppression in subjects at increased risk of infections.***
- ***Treatment for PIRD are directed at the specific genetic defect, and HCT can be a curative therapy for some cases***

References

1. Tangye SG, Al-Herz W, Bousfiha A,Chatila T, Rundles CC, Etzioni, et al. Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 2020; 40:24-64.
2. Chan AY, Torgerson TR. Primary immune regulatory disorders: a growing universe of immune dysregulation. Curr Opin Allergy Clin Immunol 2020; 20(6):582-590.
3. Chandrakasan S, Chandra S, Davila Saldana BJ, Torgerson TR, Buchbinder D. Primary immune regulatory disorders for the pediatric hematologist and oncologist: A case-based review. Pediatr Blood Cancer 2019; 66(5):e27619.

4. Matson DR, Yang DT. Autoimmune Lymphoproliferative Syndrome: An Overview. Arch Pathol Lab Med 2020 Feb;144(2):245-251.
5. Oliveira JB. The expanding spectrum of the autoimmune lymphoproliferative syndromes. Curr Opin Pediatr 2013; 25:722-729.
6. Takagi M, Shinoda K, Piao J, Mitsuiki N, Takagi M, Matsuda K, et al. Autoimmune lymphoproliferative syndrome-like disease with somatic KRAS mutation. Blood 2011; 117:2887-2890.
7. Nabhani S, Schipp C, Miskin H, Levin C, Postovsky S, Dujovny T, et al. STAT3 gain-of-function mutations associated with autoimmune lymphoproliferative syndrome like disease deregulate lymphocyte apoptosis and can be targeted by BH3 mimetic compounds. Clin Immunol 2017; 181:32-42.
8. Park JH, Lee KH, Jeon B, Ochs HD, Lee JS, Gee Hy, et al. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome: A systematic review. Autoimmun Rev 2020; 19(6):102526.
9. Verbsky JW, Chatila TA. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) and IPEX-related disorders: an evolving web of heritable autoimmune diseases. Curr Opin Pediatr 2013; 25: 708-714.
10. Zhang Y, Ma CA, Lawrence MG, Break TJ, O'Connell MP, Lyons JJ, et al. PD-L1 up-regulation restrains Th17 cell differentiation in STAT3 loss-and STAT1gain-of-function patients. J Exp Med 2017; 214:2523-2533.
11. Yazdani R, Habibi S, Sharifi L, Azizi G, Abolhassani H, Olbrich P, et al. Common Variable Immunodeficiency: Epidemiology, Pathogenesis, Clinical Manifestations, Diagnosis, Classification, and Management. J Investig Allergol Clin Immunol 2020; 30(1):14-34.
12. Bergsten E, Horne A, Arico M, Astigarraga I, Egeler RM, Filipovich AH, et al. ´Confirmed efficacy of etoposide and dexamethasone in HLH treatment: long-term results of the cooperative HLH-2004 study. Blood 2017; 130(25):2728-2738.
13. Ehl S, Astigarraga I, von Bahr Greenwood T, Hines M, Horne A, Ishii E, et al. Recommendations for the use of etoposide-based therapy and bone marrow transplantation for the treatment of HLH: consensus statements by the HLH Steering Committee of the Histiocyte Society. J Allergy Clin Immunol Pract 2018; 6(5):1508-1517
14. Chellapandian D, Das R, Zelley K, Wiener SJ, Zhao H, Teachey DT, et al. EBV-HLH Rituximab Study Group. Treatment of Epstein Barr virus-induced haemophagocytic lymphohistiocytosis with rituximab-containing chemo-immunotherapeutic regimens. Br J Haematol 2013; 162(3):376-382
15. Walter JE, Farmer JR, Foldvari Z, Torgerson TR, Cooper MA. Mechanism-based strategies for the management of autoimmunity and immune dysregulation in primary immunodeficiencies. J Allergy Clin Immunol Pract 2016; 4:1089-1100
16. Bakhtiar S, Fekadu J, Seidel MG, Gambinieri E. Allogeneic hematopoietic stem cell transplantation for congenital immune dysregulatory disorders. Front Pediatr 2019; 7:461.

CLIPPINGS

Tachypnoea is a good predictor of hypoxia in acutely ill infants under 2 months.

To evaluate the respiratory rate as an indicator of hypoxia in infants <2 months of age was the objective. Study conducted in pediatric emergency unit of an urban teaching hospital with 200 infants under 2 months, with symptom(s) of any acute illness.

Respiratory rate (by observation method), and oxygen saturation by means of a pulse oximeter were recorded at admission. Infants were categorized by presence or absence of hypoxia (SaO(2)</= 90%).

The respiratory rate was >/= 50/min in 120 (60%), >/= 60/min in 101 (50. 5%), and >/= 70/min in 58 (29%) infants. Hypoxia (SaO(2)</= 90%) was seen in 77 (38.5%) infants. Respiratory rate and SaO(2) showed a significant negative correlation (r = -0.39). Respiratory rate >/= 60/min predicted hypoxia with 80% sensitivity and 68% specificity.

These results indicate that a respiratory rate> = 60/min is a good predictor of hypoxia in infants under 2 months of age brought to the emergency service for any symptom(s) of acute illness.

Rajesh VT, Singhi S, Kataria S. Tachypnoea is a good predictor of hypoxia in acutely ill infants under 2 months. Arch Dis Child. 2000 Jan;82(1):46-9. doi: 10.1136/adc.82.1.46. PMID: 10630912; PMCID: PMC1718185.

IMMUNOLOGY

UTILITY OF GENETIC TESTS IN PRIMARY IMMUNODEFICIENCY DISORDERS

***Abhinav Jain**
****Vinod Scaria**

Abstract: *Genetic testing plays a crucial role in the field of primary immunodeficiency. It provides the confirmatory molecular diagnosis to the affected patient. This helps the family in prenatal diagnosis, personalized treatment, embryo implantation during in-vitro fertilization and family screening. In this review, we have broadly discussed the widely used genetic tests in the clinical setting for primary immunodeficiency. We have also described the most appropriate genetic testing approach for different types of primary immunodeficiency. the utility of genetic testing to the affected patients and their family members is also discussed.*

Keywords: *Primary immunodeficiency, Genetic testing, Molecular diagnosis, Personalized treatment.*

Primary immunodeficiency disorders (PIDs) are a heterogeneous group of disorders which manifest as severe or recurrent infections, autoimmune conditions, allergy, auto inflammation and malignancy. This group of disorders is caused by pathogenic genetic variants in the genes involved with the development, maturation, and activation of the immune system. The first gene identified in PID was Bruton Tyrosine Kinase (BTK) in 1993 i.e. after 41 years of diagnosis of the first case of X-linked agammaglobulinemia (XLA).[1] Since then, advancement in next generation sequencing has led to the identification of more than 400 genetically characterized PIDs and most have an autosomal recessive mode of inheritance. These PIDs have been systematically characterized by the International Union of Immunological Societies (IUIS) into ten categories based on the type of immune cell affected.[2] (Kindly refer to chapter on Clinical Immunology in the same issue of the journal). It has been estimated that more than 6 million individuals are affected by PID worldwide with the prevalence estimated to be 1:1200.[3] Due to the disease heterogeneity and lack of awareness among clinicians, approximately 90% of the patients with PID remain undiagnosed.[4]

Genetic testing plays a crucial role in confirming the diagnosis as well as in understanding the disease etiology as it has also redefined the PIDs as a heterogeneous group of disorders that were once considered as Mendelian, monogenic, and completely penetrant disorders. The identification of the genetic diagnosis helps clinicians in tailoring the treatment according to the patient's condition. It also helps in the identification of carriers or diseased individuals in the family or for community screening. It enables prenatal diagnosis and helps the family in following professional advice given during genetic counselling. Genetic testing also helps researchers in identifying novel genes and variants that are implicated in PIDs.

Genetic testing

There are different genetic tests that could be offered to the patients are given in Box 1.

Depending on the type of PID, clinical and laboratory investigations, test strengths and limitations, one could deduce what type of test would be best to genetically diagnose the patient.

Box 1. Different types of genetic tests

1. Karyotyping and fluorescent in situ hybridization (FISH)
2. Chromosomal microarray analysis (CMA)
3. Single gene test
4. Targeted gene panel (TGP)
5. Whole-exome sequencing (WES)
6. Whole-genome sequencing (WGS).

* Senior Research Fellow

** Principal Scientist,
CSIR-Institute of Genomics and Integrative Biology,
Mathura Road, New Delhi and
Academy of Scientific and Innovative Research (AcSIR),
Ghaziabad, Uttar Pradesh.

email: abhinavjj@gmail.com

Karyotyping and fluorescent in situ hybridization (FISH)

Initially, the genetic disorders were diagnosed with the help of karyotyping and FISH. Karyotyping is a process where pairing of the chromosomes is performed by visualizing under the microscope to identify chromosomal structural abnormalities. This technique can diagnose patients with large chromosomal abnormalities including Down syndrome (trisomy 21), and Wolf-Hirschhorn syndrome that are also syndromic causes of immunodeficiency. The advanced technique FISH uses fluorescent microscopy to detect the chromosomal loci specific oligonucleotide that is hybridized to its complementary DNA. FISH can detect microdeletions and duplications under 5MB that is not possible to detect using karyotyping. FISH has been widely used to diagnose Di George syndrome (DGS) i.e. microdeletion in chromosome 22q11.

Chromosomal microarray analysis

Chromosomal microarrays (CMAs) are performed through array comparative genomic hybridization (aCGH) or single nucleotide polymorphism (SNP) array hybridization. aCGH is used to detect the genomic microdeletion, microduplication i.e. copy number variant (CNVs) as well as unbalanced genomic rearrangements (translocations) by comparing the hybridization intensities between the control's and patient's DNA. CMAs cannot identify chromosomal abnormalities with no change in copy numbers like translocations and inversions. SNP array hybridization used to detect the region with the runs of homozygosity that could help to prioritize the chromosomal loci that harbour the causal variant.

It is a first-tier test recommended for children affected with syndromic disorders including neurodevelopmental delay, intellectual disabilities, and dysmorphism. In the case of PID, it is commonly used to diagnose patients affected with the 22q11 microdeletion syndrome (Di George syndrome) as well as helps in understanding the disease etiology in autosomal recessive hyper IgE syndrome. CMA also helps in the identification of the novel PID that is linked to partial trisomy 19. It identified two patients with large overlapping duplications that affects chromosome loci 19p13 and led to the diagnosis of FURID19 (facial dysmorphia, urogenital malformation, growth and neurodevelopmental retardation, immunodeficiency, trisomy 19p13).[5] Even though it provides a high resolution and sensitivity in comparison to conventional cytogenetics, it cannot resolve/identify the single nucleotide variants (SNVs) or small insertion or deletion (indels).

Single gene tests

Single gene tests (SGT) are typically performed using Sanger capillary sequencing (sequencing means a process of determining the sequence of nucleotides, Sanger sequencing is a method of DNA sequencing) and is considered as a gold standard to detect genetic variants in a single gene. Sanger sequencing was developed by Frederick Sanger and is also known as the 'chain termination method' as it uses labelled chain terminating dideoxynucleotides (ddNTPs) to terminate the DNA strand elongation where it identifies its nucleotides (A, T, G, or C). This led to the generation of the varied lengths of the DNA strands that could be sorted using gel electrophoresis and sequencing of the gene of interest. Even though it is time consuming, laborious and costly, it is highly accurate.

Capillary sequencing is widely adopted in the clinical setting for Mendelian disorders with distinct clinical characteristics. It can also be used for screening and prenatal diagnosis in families with known molecular defects. Since PID is highly heterogeneous with overlapping clinical features, a single gene test is not recommended. However, a limited number of primary immunodeficiencies are known that have distinct clinical features with the single gene disorder. Such PIDs include the X-linked agammaglobulinemia (XLA), Wiskott-Aldrich Syndrome (WAS), and hyper IgD syndrome (HIDS) that could be cost-effectively diagnosed using Sanger sequencing. However, for PIDs like severe combined immunodeficiency (SCID) and predominant antibody deficiency (PAD) that have a number of affected genes, Sanger sequencing is typically neither cost-effective, nor efficient in identifying the causal variant.

Targeted gene panels (TGP)

TGP, as the name suggests, performs the simultaneous sequencing of multiple genes implicated in a disorder. This approach covers variants in a number of genes simultaneously. This approach, based on next-generation sequencing, uses multiple probes customized to capture genomic material covering a set of predefined genes selected. The choice of the panel is typically based on the clinical characteristics and provisional diagnosis by the clinician. The probes are typically designed to capture the coding region of the genes of interest, but probes could also be designed to capture the noncoding region if known to be pathogenic in some cases. Since this approach captures a limited number of genes and genomic regions, there could be a possibility of missing out on some pathogenic genes newly implicated in the disorder. At the

same time, since TGPs offer a high depth of coverage, they can identify the single nucleotide variations (SNVs), small indels, and copy number variations (CNVs) [CNVs is one of the structural variation that include the duplication and deletion in the genome that typically of length 1Kb to 5MB]. However, in the TGP sequencing, the read depth across the genome is not uniform and the chances of the probes not getting amplified makes it quite difficult for bioinformatics pipeline to accurately detect the CNVs.

PIDs involve a large number of genes implicated in the development, activation or maturation of the T, B, NK, or innate immune cells manifesting with heterogeneous clinical features. This includes severe combined immunodeficiency (SCID), Primary antibody deficiency (PAD), and phagocyte defects that involve a large number of genes. For such PIDs, TGP would be cost-effective, as well as less labour intensive. However, there are chances of missing the variants in the newly identified genes and for obvious reasons, genes which have not yet been widely studied to be associated with PIDs. Therefore, there is also a possibility that in some patients the molecular diagnosis may be missed since there could be pathogenic variants in genes missed out by the panel. In such cases there might be a need to re sequence the patients with correct sets of genes that leads to extra cost as well as requirement for resampling of patients with uncertainty of identification of the variant.

The major limitation of the TGP is that it provides limited information about the patient genome. If novel genes were identified in the disorder, patients have to undergo sequencing to identify whether the gene harbors a causal variant. This could lead to delayed diagnosis and potentially loss of valuable time and resources.

Whole exome sequencing

Whole exome sequencing (WES) captures the coding region of the genome i.e. approximately 1-2% of the entire genomic DNA using massively parallel high throughput sequencing technology. In some cases, the WES also captures limited noncoding regions including the untranslated regions (UTRs). Similar to the TGP, WES uses probes to capture all the ~20,000 genes of the human genome. Due to its high read depth (~100X), it identifies SNVs, small indels, and CNVs throughout the entire coding region of the genome. Similar to the TGP sequencing, the WES also does not have the uniform read depth across the genome and there is a possibility of the probe not being amplified. This can lead to the inaccurate identification of the CNVs in the WES. Since WES is not limited to the number of genes, it improves the ability to identify novel genes potentially implicated in the disorder and also enables re-analysis and re-evaluation whenever new genes are identified for a particular disorder. The limitation of WES is that it cannot identify the causal variant that lies in the non-coding region (with exceptions), and is unable to accurately identify structural variants (SVs) i.e. repeat expansion, large deletion, duplication, insertion, and translocations thereby the coverage of genes remains inadequate. Due to the large amount of data, it is difficult to store and interpret data that increases the turnaround time for identification of the causal variant.

Whole genome sequencing (WGS)

Despite the advancement in next generation sequencing (NGS) technology, WES or TGP has a diagnostic yield of 30-40%.[6] There are still 60-70% of patients with PID lacking diagnosis that could be due to a causal variant that lies in the uncovered region, data analysis, variant interpretation, or understanding of PIDs. The diagnostic yield could be improved by WGS that provides a complete coverage of the patient's genome with good read depth. WGS is able to identify single nucleotide variant (SNVs), small indels, copy number variations (CNVs), as well as structural variants (SVs) with high sensitivity and specificity. Genome sequencing has been used in a recent study for 1,318 sporadic patients with primary immunodeficiency diseases (PIDD) and identified 10.3% of patients with monogenic PIDs. They also identified novel genes associated with PIDs such as influenza virus NS1-binding protein (IVNS1ABP).[7] Patients affected by PIDs also present with different phenotypes for the same mutation, due to the presence of the modifier variant that lies in the non-coding region. A WGS study of a patient with lipopolysaccharide-responsive and beige-like anchor protein (LRBA) deficiency also identified a homozygous mutation in the Nei endonuclease VIII-like 3 (NEIL3) gene involved in the base excision repair mechanism. The authors propose that both LRBA and NEIL3 deficiencies are responsible for the severity of disease in patients.[8] Although with technological advances the cost for WGS has been drastically reduced, other limitations need to be considered i.e. large amounts of data, expertise in analyzing it, and a computational intensive system. However due to these limitations WGS is not widely used in the clinical setting, but studies are underway in the PID field using WGS to understand the genetic etiologies as well as to increase our scientific understanding. The comparison of different genetic tests has been tabulated in Table I.

Table I. Comparison of different types of genetic tests.

	Single gene tests	Targeted gene panels	Whole exome sequencing	Whole genome sequencing
Used for	Single gene disorders	Diseases where multiple genes are implicated	Heterogeneous disorders	Undiagnosed genetic diseases
Coverage	Single gene	Multiple genes	Protein coding genes	Whole genome
When to use	Clinical diagnosis is accurate	Clinical diagnosis is near accurate	Clinical diagnosis is difficult	Typically when whole exome sequencing is negative
Why to use	Cost and time effective, highly accurate	Cost and time effective	Identification of novel genes	Structural and non-coding variants could be identified
Limitations	Covers only one gene, poor coverage at guanine-cytosine(GC) rich DNA region	Only well-known genes are covered	Covers only protein-coding exons and time consuming during analysis	Expensive, computationally intensive and time consuming

Utility of genetic testing

The fundamental laws of inheritance and understanding of genetics was postulated by Gregor Mendel through his experiments on pea plants in 1865.[9] These experiments provided a basic understanding of the transfer of traits from the parents to the off-spring. Similarly in case of Mendelian disorders such as PIDs, the identification of the causal pathogenic variants in the genes responsible for the disorder have a wide-ranging utility from family to population level. These varied uses are described below and well represented in Fig.1.

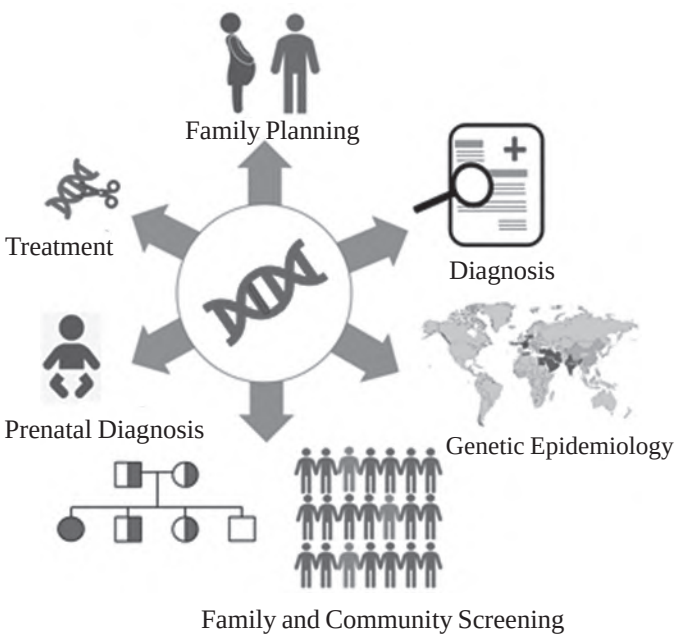


Fig.1. Utility of genetic testing for patients affected with primary immunodeficiency disorders

Genetic diagnosis

PID is a highly heterogeneous group of disorders with complex overlapping clinical features. Due to the heterogeneity of the disease, approximately 90% of the patients affected with PID remain undiagnosed.[4] Even with the advancements in clinical recognition and laboratory investigations, it takes an average of 9.2 years to diagnose a patient with PID.[10] A number of misdiagnoses and unwarranted treatments for these diseases have serious consequences on the person's health, and results in poor outcomes and permanent organ impairment. This also has psychosocial and economic implications for affected families. Sequencing can help in identifying the causal variant in patients affected with PID thus leading to a confirmatory diagnosis. This also helps in avoiding harmful and burdensome medical and surgical therapies.

Prenatal diagnosis

Approximately 3 to 5% of the fetuses are at risk of birth defects or genetic disorders in the general population.[11] However, if the disorder is identified to be genetically determined in a family, the probability of inheriting the disorder increases from 25% to 50%. Hence, the

identification of the causal variant in the family is very crucial as prenatal diagnosis could be performed. Earlier in the mid 20th century, prenatal diagnosis was performed through ultrasonography allowing evaluation of pregnancy in the early gestation stage. Now-a-days two methods are widely used i.e. amniocentesis and chorionic villus sampling (CVS) for collection of the fetal cells that aids in genetic diagnosis in the fetus. Recently the cell-free non-invasive prenatal testing is used to identify chromosomal aneuploidy, but it could not identify single gene disorders due to the challenges in separation of maternal blood from fetal blood. In case of PIDs, identification of the causal variant in the fetus either in heterozygous or homozygous genotype led to the family being able to access genetic counselling. Based on the type of PID, proactive measures are planned for the proper treatment post delivery to reduce the infant mortality or in extreme cases, with the informed consent from the family the fetus is aborted.

Family and community screening

The identification of variants in a proband could be helpful in identifying carriers or potentially affected individuals in a family. The causal pathogenic variant can be identified in the proband by approaches like WES or WGS that are quite expensive. However, after identification of the causal variant, family screening could be performed by cost-effective techniques like Sanger capillary sequencing for single nucleotide variants and small INDELs or Multiplex Ligation-dependent Probe Amplification (MLPA) for copy number variations. Once family members are identified to be carriers of the variant implicated in the disorder, appropriate evidence based genetic counselling could be provided. Genomic analysis and counselling also opens up the possibility of identification of incidental and actionable genetic variants that would have an effect on the individual's health at a later stage of life. In addition, there are variants with variable expressivity and incomplete penetrance that makes genetic counselling substantially difficult. A well-known variant p.R117H in cystic fibrosis transmembrane conductance regulator (CFTR) gene implicated in cystic fibrosis was found to have low penetrance and was suggested to be removed from the newborn screening panel.[12]

The community or population screening could also be an opportunity for enabling evidence based genetic counselling for variants of high allele frequency in a specific community or population. A number of causal variants in small endogamous communities are inherited from the founder of the population and are popularly known as founder variants. These founder variants arise either due to the bottleneck effect or founder effect. In bottleneck effect, a large proportion of the population get drastically reduced due to the environmental catastrophe, habitat destruction or mass genocide of a specific community whereas in the founder effect, few individuals from the population get isolated and form a new population. There are few populations that underwent bottleneck or founder effect that includes Finnish, Middle Eastern and Ashkenazi Jewish population. These population had prevalent disease causing founder variants that led to the population and newborn screening of these variants in these populations.[13] Identification of the founder variant has a potential to improve the healthcare facilities of the population and improve the medical condition of the patients.[14] In case of the PIDs, the majority of founder variants are implicated in the autoinflammatory disorders in which the five most common founder variants are V726A, M694V, M694I, M680I and E148Q in the MEFV gene implicated in Familial Mediterranean Fever (FMF) that accounts for 74% of the variants in the Armenian, Arab, Jewish, and Turkish population.[15]

Genetic epidemiology

Despite the advancements in the healthcare sector such as potent drugs, vaccinations, medical facilities, appropriate diet, and proper hygiene, more than 5 million children under the age of 5 years die annually.[16] Infectious diseases are still a major cause of deaths in this age-group including pneumonia, and diarrhea. It is also noteworthy that a small proportion of these patients may have underlying primary immunodeficiency disorders. The prevalence of PID is considered to be 1 in 10,000 symptomatic individuals worldwide which is an underestimation due to the misdiagnosis of patients with PID, limited diagnostic facilities, and lack of awareness among clinicians and lack of properly maintained PID registries.[17] The proper maintenance of a registry for patients with PID will help estimate the prevalence of the type of PID across the globe. Accordingly, the governments can formulate policies for screening, management and treatment required. Another method that could be adopted for assessing the prevalence of PID in the country is by sequencing a number of healthy individuals of different geographical regions or ancestries from all over the country.[18, 19] Genome sequencing will help uncover the genetic architecture of the population that will help in the identification of individuals who are carriers for genetic variants implicated in PID. The frequency of the variants in the population will help identify the genetic epidemiology of the PID in the population as well as help in the identification of the founder variants

Genetic Screening and Infant Selection

Genetic testing plays a crucial role in family planning for the known causal variant implicated in the PID. In order to avoid child being born with a disease causing genetic variant, the couples could opt for in-vitro fertilization (IVF). In IVF, they can choose oocytes and sperm without the causal variant to establish pregnancy. Genetic testing could also be considered in the future to identify a causal variant in couples before marriage to avoid any chances of affected offspring.

Treatment

Determining the molecular cause of PID would impact the patient's condition as well as be helpful in making a right decision for a therapeutic approach. One of the invariably fatal PIDs is SCID that has more than 15 types and manifest similar clinical symptoms. It is of prime importance to identify the patients suffering from SCID in early periods of life, so that patients can undergo hematopoietic stem cell transplantation (HSCT). HSCT is the most tried and trusted therapeutic approach for the SCID patients. But due to the variable outcomes and strategies for HSCT in different types of SCID, it is very crucial to understand the genetic cause of the disease. Patients with SCID who had defects in the genes Recombination Activating 1,2 (RAG1,RAG2) or adenosine deaminase (ADA) had poorer T cell engraftment after hematopoietic stem-cell transplantation (HSCT), but patients with defects in Janus Kinase 3 (JAK3) had poor B cell engraftment after HSCT.[20] It is critical to assess patients with failure of the engraftment of different lymphoid cells after the transplantation. The T-B+SCID has a functional B cell and could discontinue the immunoglobulin supplementation after HSCT. Patients with SCIDs who had genetic defects in DNA ligase 4 (LIG4), non-homologous end joining factor 1 (NHEJ1), and Nibrin (NBS1) have been shown to have high mortality following conditioning provided prior to the transplantation. Patients with RAG1 and RAG2 variants would have high risk for DNA damage on exposure to the Busulfan and ionizing radiation.[21] Thus HSCT for different types of SCID should be considered with the prior knowledge of the defective gene. Even though HSCT is considered as a curative treatment, there is a high risk of rejection and other complications associated with it. Recently gene therapy has been trialled as a therapeutic option and shown to be successful for various PIDs including SCID, Wiskott-Aldrich syndrome (WAS), and chronic granulomatous disease (CGD). Gene therapy has significantly improved its biosafety using sophisticated tools like lentiviral vectors and clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR-associated (Cas9).

There are PIDs that could be treated with medications, but genetic diagnosis is crucial as in the case of ADA SCID that is due to the lack of enzyme adenosine deaminase. enzyme replacement therapy could serve as bridge therapy and correct the metabolic derangements until HSCT or gene therapy for patients affected with ADA SCID. Similarly for cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) haplo insufficiency and the signal transducer and activator of transcription 3 (STAT3) gain of function variant could be treated with rapamycin. Patients affected with common variable immunodeficiency (CVID) with gene defect in LRBA respond to biologic therapy with abatacept. There are various medications available that could provide definitive treatment for patients with PID only when the genetic diagnosis is known. We hope that identification of the genetic defects in PIDs would influence targeted and personalized therapeutic developments.

Conclusion

Due to the heterogeneity of the clinical presentations of PIDs, a significant number of patients remain undiagnosed, while early molecular diagnosis could have enabled appropriate treatment and prevented mortality in a significant number of patients. Given the resource constraints for molecular diagnosis, it is of prime importance to choose the appropriate genetic testing method based on the clinical characteristics of the patients. Genetic testing provides a great boon for patients affected with PID that includes personalized treatment, family or community screening, prenatal diagnosis and family planning. With the advancement in sequencing technology and clear advantage to the patients, we hope that in the near future molecular diagnosis will be considered as a first tier of diagnosis in the field of PIDs.

Points to Remember

- ***Genetic testing provides the confirmatory diagnosis for the patients affected with primary immunodeficiency that has a heterogeneous array of symptoms.***
- ***The identification of the variant helps the clinicians in tailoring the treatment of the patient according to the genetic condition.***
- ***Variant identification helps in prenatal diagnosis, embryo pre implantation, family and community screening.***
- ***Choosing the most appropriate genetic test for diagnosis of different types of PID is based on the patient's clinical characteristics and immunological investigations.***

References

1. Vetrie D, Vorechovský I, Sideras P, Holland J, Davies A, Flinter F, et al. The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. Nature 1993; 361(6409):226-233.
2. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 2020; 40(1):24-64.
3. Boyle JM, Buckley RH. Population prevalence of diagnosed primary immunodeficiency diseases in the United States. J Clin Immunol 2007; 27(5):497-502.
4. World PI Week. What is Primary Immunodeficiency (PI)? [Internet]. Available from: http://www.worldpiweek.org/what-is-primary-immunodeficiency-pi/Accessed on 12th April 2021.
5. Seidel MG, Duerr C, Woutsas S, Schwerin-Nagel A, Sadeghi K, Neesen J, et al. A novel immunodeficiency syndrome associated with partial trisomy 19p13. J Med Genet 2014; 51(4):254-263.
6. Stray-Pedersen A, Sorte HS, Samarakoon P, Gambin T, Chinn IK, Coban Akdemir ZH, et al. Primary immunodeficiency diseases: Genomic approaches delineate heterogeneous Mendelian disorders. J Allergy Clin Immunol 2017; 139(1):232-245.
7. Thaventhiran JED, Lango Allen H, Burren OS, Rae W, Greene D, Staples E, et al. Whole-genome sequencing of a sporadic primary immunodeficiency cohort. Nature 2020; 583(7814):90-95.
8. Massaad MJ, Zhou J, Tsuchimoto D, Chou J, Jabara H, Janssen E, et al. Deficiency of base excision repair enzyme NEIL3 drives increased predisposition to autoimmunity. J Clin Invest 2016; 126(11):4219-4236.
9. Abbott S, Fairbanks DJ. Experiments on Plant Hybrids by Gregor Mendel. Genetics. 2016; 204(2):407-422.
10. Immune Deficiency Foundation (IDF). Primary immunodeficiency diseases in America: 2007. Available from: https://primaryimmune.org/wp-content/uploads/2011/04/Number-59-Fall-2008.pdf. Accessed on 12th April 2021.
11. Centers for Disease Control and Prevention (CDC). Update on overall prevalence of major birth defects-Atlanta, Georgia, 1978-2005. MMWR Morb Mortal Wkly Rep 2008; 57(1):1-5. Available from https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5701a2.htm. Assessed on 12th April 2021.
12. Thauvin-Robinet C, Munck A, Huet F, Génin E, Bellis G, Gautier E, et al. The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counselling and newborn screening. J Med Genet 2009; 46(11):752-758.
13. Manchanda R, Loggenberg K, Sanderson S, Burnell M, Wardle J, Gessler S, et al. Population testing for cancer predisposing BRCA1/BRCA2 mutations in the Ashkenazi-Jewish community: a randomized controlled trial. J Natl Cancer Inst 2015; 107(1):379.
14. Jain A, Sharma D, Bajaj A, Gupta V Scaria V.Founder variants and population genomes-Toward precision medicine. Adv Genet 2021; 107:p.121-152.
15. Touitou I. The spectrum of Familial Mediterranean Fever (FMF) mutations. Eur J Hum Genet 2001; 9(7):473-483.
16. World Health Organization. Children: improving survival and well-being https://www.who.int/news-room/fact-sheets/detail/children-reducing-mortality. Accessed on 24th Mar 2021.
17. Bousfiha AA, Jeddane L, El Hafidi N, Benajiba N, Rada N, El Bakkouri J, et al. First report on the Moroccan registry of primary immunodeficiencies: 15 years of experience (1998-2012). J Clin Immunol 2014; 34(4): 459-468.
18. Jain A, Bhoyar RC, Pandhare K, Mishra A, Sharma D, Imran M, et al. IndiGenomes: a comprehensive resource of genetic variants from over 1000 Indian genomes. Nucleic Acids Res 2021; 49(D1):D1225-1232.
19. Jain A, Gandhi S, Koshy R, Scaria V. Incidental and clinically actionable genetic variants in 1005 whole exomes and genomes from Qatar. Mol Genet Genomics 2018; 293(4):919-929.
20. Buckley RH, Win CM, Moser BK, Parrott RE, Sajaroff E, Sarzotti-Kelsoe M. Post-transplantation B cell function in different molecular types of SCID. J Clin Immunol 2013; 33(1):96-110.
21. Cowan MJ, Gennery AR. Radiation-sensitive severe combined immunodeficiency: The arguments for and against conditioning before hematopoietic cell transplantation-what to do?. J Allergy Clin Immunol 2015; 136(5):1178-1185.

GLOSSARY IN IMMUNOLOGY

Multiplex ligation-dependent probe amplification (MLPA): It is a variation of the multiplex polymerase chain reaction that permits amplification of multiple targets with only a single primer pair. It is a method for detecting copy number changes using PCR. MLPA is unique in that it can detect copy number changes in up to 50 different DNA sequences in a single reaction, and it can distinguish sequences differing by only one nucleotide.

IMMUNOLOGY

FOOD DEPENDENT EXERCISE INDUCED ANAPHYLAXIS

Major K. Nagaraju

Abstract: *Food dependent exercise induced anaphylaxis is an uncommon condition in childhood and occurs during exercise, preceded by ingestion of culprit food, which used to be independently tolerated. Wheat gluten is the commonest food responsible for food dependent exercise induced anaphylaxis. Diagnosis is mainly by evaluation of clinical history. As allergy tests do not give accurate results, modified exercise challenge tests are needed. Accurate diagnosis definitely helps the patient to return to exercise with confidence. Patient should avoid exercise for 4-6 hours after consuming the offending food but can take other foods without any restriction. Parents should be educated about the importance of carrying epinephrine for emergency.*

Keywords: *Food dependent exercise induced anaphylaxis, Cofactors, Challenge test, Wheat dependent.*

Maulitz et al described a case of shellfish induced anaphylaxis in 1979 triggered by exercise.[1] Thereafter it was labelled as food dependent exercise induced anaphylaxis (FDEIA). In such cases anaphylaxis occurs within 2-4 hours after exercise preceded by ingestion of trigger food. Most of the people develop symptoms after intake of solid food rather than liquid food. IgE antibodies are demonstrable to the food substance which causes the anaphylaxis, but no reaction occurs without exercise after food ingestion. Majority of patients may present with the first episode of anaphylaxis even after years of routine exercises.[2] Manifestations of the allergic symptoms often depend on the amount of food ingested. Many cases require strenuous exercise for the precipitation of symptoms. However, in some cases trivial activity also suffices.

* Professor of Pediatrics,
Division of Allergy and Clinical Immunology,
Saveetha Medical College and Hospital,
Chennai.

email : majorknr@yahoo.in

Epidemiology

FDEIA is rare, but reported from all parts of the world. It is more commonly seen in adolescents and young adults, but reported in all age groups. Japan reports prevalence of about 0.017% in high school children.[3] Prevalence of FDEIA is increasing worldwide. In India except for few case reports especially in adolescents and adults, no proper data is available in pediatric population. This condition is probably under diagnosed and needs proper a history to be identified.

Types of exercise induced anaphylaxis (EIA)

Two types of EIA are described (Fig.1). i) Nonfood dependent exercise induced anaphylaxis (NFDEIA) and ii) food dependent exercise induced anaphylaxis. FDEIA is further classified into food specific and nonfood specific. In NFDEIA, anaphylaxis can occur within few minutes to hours after consumption of any food (not specific) followed by exercise, whereas in FDEIA, anaphylaxis occurs only after intake of a specific food prior to the exercise.[2]

Pathophysiology

Pathophysiology of FDEIA is not fully understood, but several theories are suggested by researchers which are as follows: i) most recent one is that, during physical activity, there is increased gastric permeability, leading to greater access of allergenic proteins to the gut associated immune system. ii) mast cell degradation occurs due to alteration of plasma pH after exercise iii) increased cross linking of the IgE due to stimulation of tissue transglutaminase enzyme during physical activity iv) changes in the plasma osmolality due to exercise.[4] Barg et al demonstrated an increase in histamine release in FDEIA at 340 mosm, but not at 280 or 450 mosm.[5] Cooper et al reported that during exercise, the circulating leukocytes are activated and stimulate the pro inflammatory response.[6] v) during exercise, blood is shunted to muscles and skin, leading to displacement of ingested food proteins from the gut, causing anaphylaxis. It is proposed that during exercise, threshold for food allergy gets lowered.[7]

Triggering factors

Common exercises that trigger FDEIA are jogging,

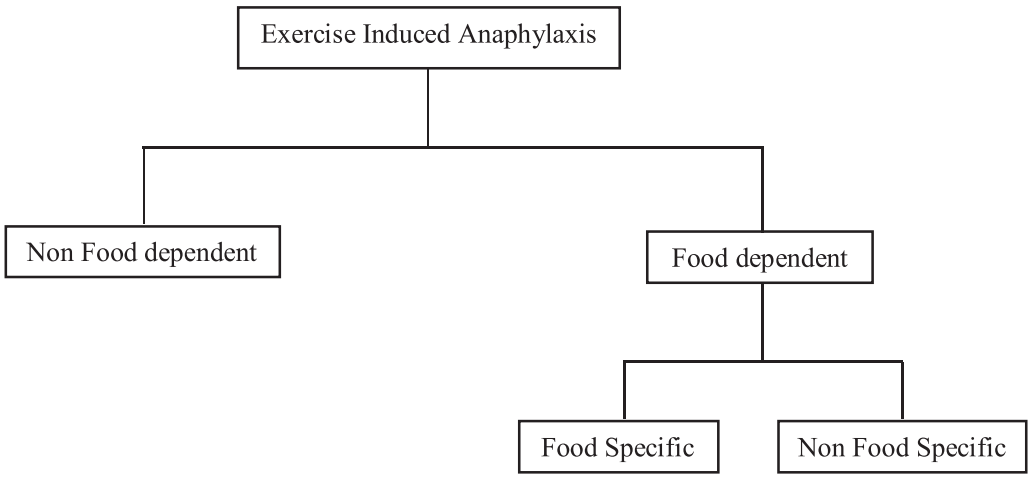


Fig.1. Classification of exercise induced anaphylaxis

cycling, running, dancing and sport activities. Though exercise initiates symptoms of FDEIA, several cofactors can reduce the threshold.[8] Several authors have reported that other factors can initiate the symptoms of anaphylaxis like cold, fatigue, sleep deprivation and in some patients only during winter. If cofactors exaggerate the symptoms, it is called "Food dependent co factor augmented anaphylaxis".[8]

Food substances responsible for FDEIA

Any food can cause FDEIA, but it is different from region to region. Commonest food responsible for FDEIA is wheat gluten. Other foods implicated are shrimp, peanut, fish, pork, beef, nuts, tomatoes, mushrooms, eggs, peaches, apples and milk.

There are some case reports with ingestion of two incriminated foods together triggering FDEIA along with exercise. Country wise foods triggering FDEIA are mentioned in Table I.

Cofactors

Several cofactors are implicated in developing a reaction in FDEIA patients like alcohol, menstrual cycle, cold and warm environment, NSAIDs, atopic dermatitis and humidity.[3] Quintela et al demonstrated that ingestion of alcohol can trigger high total IgE levels.[7] In some cases multiple cofactor combination may be required to develop the symptoms. The cofactors responsible for FDEIA are mentioned in Box 1.

Clinical features

Most patients with FDEIA initially experience dermatologic symptoms like intense pruritus, urticaria with or without angioedema. Few patients present with syncope, wheeze, cough or hypotension. Most symptoms usually appear within one hour of the exercise. Rosier et al report a case of allergic myocardial infarction (Kounis syndrome) with FDEIA in a 49 year old patient from Netherlands. They have documented the elevation of troponin T and creatine kinase MB with ECG changes in a patient with already established wheat dependent exercise induced anaphylaxis (WDEIA).[9]

Table I. Foods responsible for FDEIA

Country	Causative foods
Japan	Wheat, shrimp, mushroom, rice
Italy	Wheat, tomatoes & other vegetables
Germany	Wheat
Korea	Wheat, shrimp, apple
India	Wheat, chickpea, orange
USA	Wheat, shellfish, peanuts, celery
Thailand	Wheat
Sri Lanka	Wheat
Canada	Wheat, chickpea
Middle East	Wheat, peanut

Diagnostic criteria for FDEIA[2]

1. Clinical diagnosis of anaphylaxis according to world allergy organization (WAO) criteria
2. Symptoms must appear within 4 hours of ingestion of suspected food and the patient has history of definitive exertion.

Box 1. Cofactors for FDEIA

- NSAIDs
- Alcohol
- Stress
- Infections
- Premenstrual and menstrual phase
- Opioids
- Sleep deprivation (rarely)
- Antacids
- Beta blockers
- Angiotensin converting enzyme inhibitors

3. If patient does not physically exert within 4-6 hours after consuming causative food, the symptoms do not appear.
4. Demonstration of specific IgE positivity to causative food either by skin prick test (SPT) or by serum specific IgE.

Clinical history

FDEIA is mainly diagnosed by clinical history and exclusion of other disorders as in Table II.

The following allergy questionnaire can help to establish the diagnosis of FDEIA[10]

1. Do the symptoms occur with each episode of exercise after ingestion of offending food?
2. Recall of food which was taken 2-4 hours before the exercise.
3. Do similar symptoms occur without food intake and with exercise?
4. Does the patient feel that a particular diet might have caused these symptoms?
5. Has the patient consumed any medicines before the episode and if so what medicines?
6. Does the patient suffer from any other allergic diseases like allergic rhinitis, urticaria or asthma? If so, are they under control ?
7. Is there any history of consumption of alcohol prior to the episode, especially in adolescents / adults.

Skin prick test

SPT can help to identify the suspected food allergen. Commercially available extracts are not accurate enough to diagnose FDEIA. For wheat, the sensitivity and specificity were reported to be 40% and 74% respectively. Prick to prick technique using fresh foods described by Dreborg and Foucard can be used if allergens are not available commercially.[11]

Measurement of serum specific IgE to the causative food may help. But accuracy may not be good and diagnosis may be missed. By analysing the wheat protein components it is revealed that about 80% of the patients with WDEIA react to omega 5 gliadin and the remaining 20% patients react to high molecular weight glutenin (HMW-glutenin). It is important to measure both omega 5 gliadin and HMW-glutenin in WDEIA.[12] It is advisable to perform SPT with gliadin extracts to detect omega 5 gliadin sensitisation rather than whole wheat commercial extract, mainly due to the low content of omega 5 gliadin in the latter. Serum tryptase levels are elevated when measured within 6 hours of anaphylaxis and normalise in 48 hours.

Table II. Differential diagnosis of FDEIA

Disease condition	Features
Cholinergic urticaria	Unrelated to food. Due to increased body temperature, often associated with exercise. Pin point urticaria(Cholinergic urticaria presents as itchy pin point and non follicular)
Exercise induced anaphylaxis	Unrelated to food, triggered by exercise. (During exercise, endogenous endorphins are released. Endorphins are known to be mast cell secretagogues).
Food allergy (IgE mediated)	Related to ingestion of food, no relation to exercise.
Hereditary angioedema	No urticaria; low serum C4 levels; maybe exacerbated by exercise. Family history elicitable
Mastocytosis	Elevated serum tryptase levels; exercise is also a trigger urticaria pigmentosa will be present.
Exercise induced asthma	No cutaneous features; triggered by exercise, not related to food ingestion.

Challenge tests

SPT combined with oral food challenges along with exercise can diagnose FDEIA. Using the cofactors like aspirin (NSAIDs) or alcohol along with exercise and oral food challenge (suspected food), symptoms can be elicited. Challenge test protocol is mentioned in Fig.2. Mahidol University, Thailand developed a 3 day modified protocol for diagnosis of FDEIA, in adults, which can be used in adolescents and older children also.[13,14] This protocol is illustrated in Fig.3. In children exercise challenge is similar to exercise induced bronchoconstriction. Children should continue the exercise for at least 6 minutes until they reach 80% of the maximum heart rate.[9] In addition child should also be clinically screened for other causes such as focal infection affecting ear, nose throat and teeth.

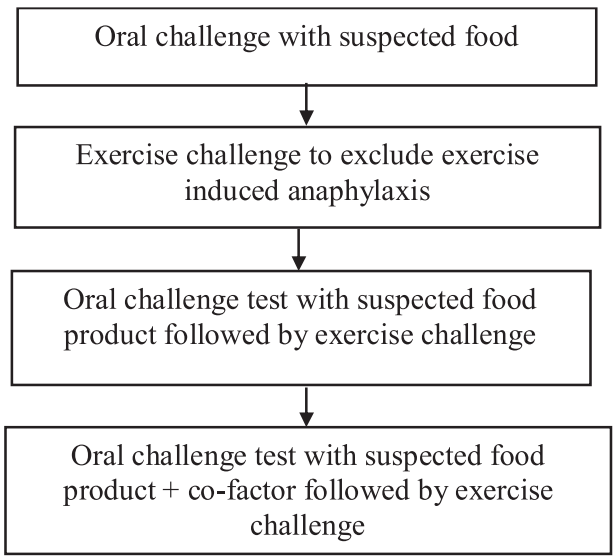


Fig.2. Protocol for challenge tests in FDEIA

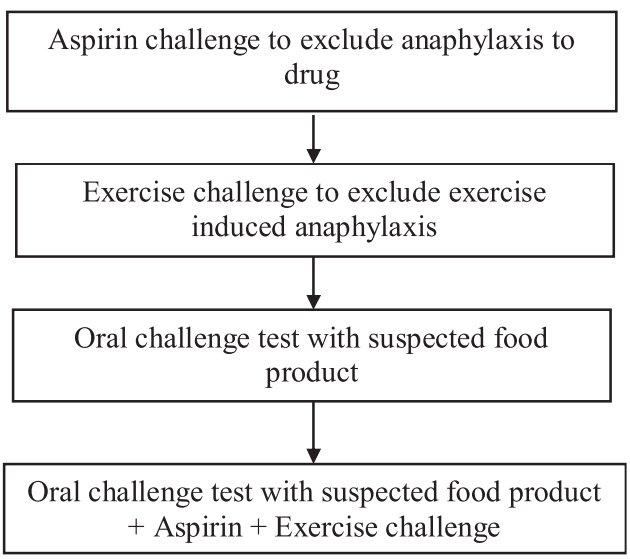


Fig.3. Modified protocol for challenge tests in FDEIA (3 day protocol)

Differential diagnosis

Most of the time, FDEIA is under diagnosed and misdiagnosed as idiopathic anaphylaxis by practising physicians. Common differential diagnosis for FDEIA are mentioned in Table II, which should be excluded.

Treatment of FDEIA

Management

Management of FDEIA includes education about safe conditions for exercise, the importance of ceasing exercise immediately if symptoms develop, appropriate use of epinephrine and avoidance of the culprit food for at least 4 hours before exercise and 1 hour after exercise.[7] Consulting a dietitian is important for dietary changes.[10] Emergency management of anaphylaxis includes administration of 1:1000 (1mg/ml) epinephrine at a calculated dose of 0.01mg/kg (maximum of 0.3 mg in children and 0.5mg in adults) intramuscularly into the anterolateral aspect of the thigh.[10] Antihistamines are to be used in controlling the skin symptoms associated with anaphylaxis.

Prevention

Avoidance of the potential precipitating food prior to exercise is of crucial importance.[10] Currently there are no approved prophylactic drugs to prevent further attacks of FDEIA, due to lack of double blind randomized trials. Only case reports are available. Sugimura et al demonstrated that in FDEIA to wheat, administration of oral disodium cromoglycate before ingestion and prior to exercise, prevented symptoms of anaphylaxis in two children. In children 100mg oral preparation (100mg/5ml) taken orally 20 minutes before meals, upto 3 times daily can prevent development of further symptoms of FDEIA,[15] but not consistent in all patients. It was observed that symptoms of FDEIA reappeared whenever children missed the dose of cromoglycate. Use of antihistamines prior to the episode is not recommended due to possible masking of early symptoms of FDEIA. Peroni et al from Italy reported that the use of montelukast with cetrizine prevented further attacks of FDEIA in a 17 year adolescent with peach allergy.[16] Bray et al reported the usefulness of 300mg of omalizumab monthly for 4 months in a 14 year old male athlete, who was having FDEIA with sensitization to multiple foods.[17]

Desensitization for the offending food is not routinely recommended. Only few case studies are available for milk and wheat. Specific oral tolerance induction (SOTI) with IFN-gamma was successfully done in 2 cases of WDEIA

in 2 adult patients by Noh et al.[18] Caminiti et al reported that return of symptoms after exercise in a 9 year old boy who had successfully undergone SOTI to milk dependent exercise induced anaphylaxis. They advise that the achievement of induced tolerance may be partial and is lost by strenuous exercise.[19]

Education of patient and parents

The patient and parents are to be educated about the disease, symptoms and signs of anaphylaxis and how to recognize these early. The patient should be taught self-administration of epinephrine. The importance of avoiding exercise at least for 4 hours after ingestion of the offending food, is to be explained. The patient must wear a medical alert bracelet mentioning about the condition.

Recommendations for patients with FDEIA

1. Should carry preloaded epinephrine or auto-injector
2. Food causing FDEIA should be avoided for 4-6 hours before exercise.
3. Common cofactors should be avoided
4. If necessary, he/she should exercise with other persons who are familiar with the patient's condition and the technique of administration of epinephrine.
5. Forced - exercise should be stopped immediately, if any symptoms occur.

Conclusion

FDEIA is a rare form of anaphylaxis, in which the patient gets symptoms after ingestion of offending food followed by physical activity. Neither exercise alone nor offending food ingestion alone causes anaphylaxis. The pathophysiology of FDEIA is not fully understood. The best way to prevent FDEIA is to avoid the offending food before 4 hours of physical activity. Specific IgE tests for offending food are not reliable. Omega 5 gliadin is the marker for wheat dependent exercise induced anaphylaxis. Exercise challenge along with ingestion of culprit food with cofactors can reveal the diagnosis. Patient must be taught about the use of self-administered adrenaline in case of emergency.

Points to Remember

- ***FDEIA is a special type of food allergy, where symptoms are triggered by consumption of causative food combined with exercise.***
- ***Pathophysiological mechanism of FDEIA is not fully understood.***
- ***Exercise tolerance test combining aspirin along with suspected food allergen can establish the diagnosis and can exclude other causes.***
- ***Omega 5 gliadin is the preferred marker for diagnosing wheat dependent exercise induced anaphylaxis.***
- ***Children with FDEIA should avoid eating the causative food 4 hours before any exercise/exertion.***

References

1. Maulitz RM, Pratt DS, Schocket AL. Exercise-induced anaphylactic reaction to shellfish. J Allergy Clin Immunol 1979; 63:433-434.
2. Robin M, Karadan U. Nonspecific food dependant exercise-induced anaphylaxis with seizure: a case report. Asian J Med Sci 2020; 11:94-97.
3. Asaumi T, Ebisawa M. How to manage food dependent exercise induced anaphylaxis (FDEIA). Curr Opin Allergy Clin Immunol 2018; 18(3):243-247.
4. Paula RA, George DT. Pathophysiology, diagnosis and management of exercise-induced anaphylaxis. Curr Opin Allergy Clin Immunol 2010; 10:312-317.
5. Barg W, Wolanczyk-Medrala A, Obojski A, Wytrychowski K, Panaszek B, Medrala W. Food-dependent exercise-induced anaphylaxis: possible impact of increased basophil histamine releasability in hyperosmolar conditions. J Investig Allergol Clin Immunol 2008; 18(4):312-315.
6. Cooper DM, Radom-Aizik S, Schwindt C, Zaldivar F Jr. Dangerous exercise: lessons learned from dysregulated inflammatory responses to physical activity. J Appl Physiol 2007; 103(2):700-709.
7. Tam CJ, John RM. Food-Dependent Exercise-Induced Anaphylaxis: A Review. J Nurse Pract 2017; 13(5):313-321
8. Christensen MJ, Eller E, Mortz CG, Brockow K, Bindslev-Jensen C. Wheat-Dependent Cofactor-Augmented Anaphylaxis: A Prospective Study of Exercise, Aspirin, and Alcohol Efficacy as Cofactors. J Allergy Clin Immunol Pract 2019; 7(1):114-121.
9. Rosier SE, Otten R, Brugts JJ, Hoek AE. Allergic acute coronary syndrome in exercise-induced anaphylaxis. Neth J Med 2018; 76(9):411-414.
10. Toit GD. Food-dependent exercise-induced anaphylaxis in childhood. Pediatr Allergy Immunol 2007; 18:455-463.
11. Morita E, Matsuo H, Chinuki Y, Takahashi H, Dahlström J, Tanaka A. Food-dependent exercise-induced anaphylaxis -importance of omega-5 gliadin and HMW-glutenin as causative antigens for wheat-dependent exercise-induced anaphylaxis. Allergol Int 2009; 58(4):493-498.
12. Thongngarm T, Wongsa C, Pacharn P, Piboonpocanun S, Sompornrattanaphan M. Clinical Characteristics and Proposed Wheat-Cofactor Challenge Protocol with a High

Diagnostic Yield in Adult-Onset IgE-Mediated Wheat Allergy. J Asthma Allergy 2020; 13:355-368.

13. Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. Allergy 1983; 38:167-172.
14. Pacharn P, Jirapongsananuruk O, Daengsuwan T, Vichyanond P, Visitsunthorn N. Wheat-dependent, exercise-induced anaphylaxis in Thai children: a report of 5 cases. Asian Pac J Allergy Immunol 2009; 27(2-3):115-120.
15. Sugimura T, Tananari Y, Ozaki Y, Maeno Y, Ito S, Yoshimoto Y, et al. Effect of oral sodium cromoglycate in 2 children with food-dependent exercise-induced anaphylaxis (FDEIA), Clin Pediatr (Phila) 2009; 48(9):945-950.
16. Peroni DG, Piacentini GL, Piazza M, Cametti E, Boner AL. Combined cetirizine-montelukast preventive treatment for food-dependent exercise-induced anaphylaxis. Ann Allergy Asthma Immunol 2010; 104(3):272-273.
17. Bray SM, Fajt ML, Petrov AA. Successful treatment of exercise-induced anaphylaxis with omalizumab. Ann Allergy Asthma Immunol 2012; 109(4):281-282.
18. Noh G, Lee JH. Specific Oral Tolerance Induction Using IFN-Gamma in 2 Cases of Food-Dependent Exercise-Induced Anaphylaxis. Case Rep Med 2013; 2013:259692.
19. Caminiti L, Passalacqua G, VitaD, Ruggeri P, BarberioG, Pajno GB. Food-exercise-induced anaphylaxis in a boy successfully desensitized to cow milk. Allergy 2007; 62(3): 335-336.

CLIPPINGS

Immunopathogenesis of COVID-19-related pediatric multisystem inflammatory syndrome and Kawasaki disease.

Multisystem inflammatory syndrome associated with the SARS-CoV-2 (MIS-C)pandemic has recently been described in children, partially overlapping with Kawasaki disease (KD).

It is hypothesized that (a) MIS-C and pre-pandemic KD cytokine profiles may be unique and justify the clinical differences observed, and (b) SARS-CoV-2-specific immune complexes (ICs) may explain the immunopathology of MIS-C.

Seventy-four children were included; 14 with MIS-C, 9 patients positive for SARS-CoV-2 by PCR without MIS-C (COVID), 14 with pre-pandemic KD, and 37 healthy controls (HCs). Thirty-four circulating cytokines were quantified in pretreatment serum or plasma samples and the presence of circulating SARS-CoV-2 ICs was evaluated in MIS-C patients.

Compared with HCs, the MIS-C and KD groups showed most cytokines to be significantly elevated, with IFN-γ-induced response markers (including IFN-γ, IL-18, and IP-10) and inflammatory monocyte activation markers (including MCP-1, IL-1α, and IL-1RA) being the main triggers of inflammation. In linear discriminant analysis, MIS-C and KD profiles overlapped; however, a subgroup of MIS-C patients (MIS-Cplus) differentiated from the remaining MIS-C patients in IFN-γ, IL-18, GM-CSF, RANTES, IP-10, IL-1α and SDF-1 and incipient signs of macrophage activation syndrome. Circulating SARS-CoV-2 ICs were not detected in MIS-C patients.

Authors concluded that the findings suggest a major role for IFN-γ in the pathogenesis of MIS-C, which may be relevant for therapeutic management.

Esteve-Sole A, AntonJ , Pino-Ramirez RM , Sanchez-Manubens J, Fumadó V , Fortuny C. Similarities and differences between the immunopathogenesis of COVID-19-related pediatric multisystem inflammatory syndrome and Kawasaki disease.J Clin Invest.2021 Mar 15;131(6):e144554. doi: 10.1172/JCI144554.

IMMUNOLOGY

HEREDITARY ANGIOEDEMA

***Archan Sil**
****Ankur K. Jindal**

Abstract: *Hereditary angioedema is an uncommon disorder with autosomal dominant mode of inheritance and is clinically characterized by recurrent episodic swelling of face, limbs, genitals, airway and gastrointestinal tract. Because of lack of awareness, most patients with hereditary angioedema remain undiagnosed and untreated. Swelling episodes in patients with hereditary angioedema are mediated by bradykinin. Excess bradykinin due to defective C1 inhibitor protein is the basic fault. While in type 1 HAE, C1 inhibitor protein levels are low, HAE type 2 is characterized by normal levels of C1 inhibitor protein that is functionally defective. C1 inhibitor protein levels. and function are normal in type 3 hereditary angioedema Treatment of acute attacks, short term prophylaxis and long-term prophylaxis are the mainstay in management. C1 inhibitor protein concentrate is the preferred treatment for patients with hereditary angioedema in the developed countries. However, because of non-availability of this drug in India and many other developing countries, most patients are treated with fresh frozen plasma, attenuated androgens and tranexamic acid. In this review, we update on the pathogenesis, clinical features, diagnosis and management of hereditary angioedema.*

Keywords: *Hereditary angioedema, Bradykinin, C1 inhibitor, Acute attacks, prophylaxis, Attenuated androgens, Tranexamic acid.*

Angioedema is characterized by swelling of deeper dermis and subcutaneous or submucosal tissues due to increased vascular permeability. It may be broadly categorized into a) histamine induced and b) bradykinin induced angioedema (Box 1). Histamine induced angioedema is characterized by sudden onset swelling, often associated with itching, may be associated with urticaria and often responds to antihistaminic drugs. On the other hand, bradykinin-induced angioedema is relatively slow in onset, not associated with itching and urticaria and do not respond to antihistaminic drugs. Bradykinin mediated angioedema may be hereditary or acquired.[1] While acquired bradykinin mediated angioedema (seen in patients with autoimmune diseases and lymphoma and also associated with few drugs such as angiotensin convertase enzyme inhibitors) often do not present in children, most cases of hereditary angioedema (HAE) have disease onset in childhood.

Box 1. Types of angioedema

1. Histamine induced angioedema: Sudden onset swelling, presence of itching and urticaria and often responds to antihistaminic drugs.

2. Bradykinin induced angioedema: Slow in onset, not associated with itching and urticaria and do not respond to antihistaminic drugs.

a) Hereditary: Onset in childhood

b) Acquired: Often do not present in children

HAE is an uncommon, potentially life-threatening disease and has an autosomal dominant mode of inheritance. This disease is characterized by repetitive and sudden episodes of subcutaneous or submucosal swelling typically affecting extremities, face, genitals, airway and gastrointestinal mucosa.[2] Clinical presentation was first described by Quincke, while Osler first recognized the autosomal dominant pattern for this disease in 1888.[3]

Because of lack of awareness, most patients with HAE remain undiagnosed and untreated. Considering the global prevalence of HAE (1:10,000 to 1:50,000) and the population of India, it is expected that there are more than 520,000 patients with HAE in India. However, at present, there are not more than 200 diagnosed patients with this disease in India. This suggests that HAE is grossly under recognized in our country. As most patients with HAE begin

* Senior Resident, (DM-Pediatric Clinical Immunology and Rheumatology),

** Assistant Professor of Pediatric Clinical Immunology and Rheumatology,
Allergy Immunology Unit, Department of Pediatrics,
Advanced Pediatrics Centre,
Post Graduate Institute of Medical Education and Research, Chandigarh.

email: ankurjindal11@gmail.com

Box 2. Classification of HAE

(1) HAE-1: Most common type (~85% of all patients) and is diagnosed by low C1-INH levels.

(2) HAE-2: Second most common type (~15% of all patients) and is diagnosed by normal C1-INH levels but low C1-INH functional activity. Both type 1 and type 2 HAE are caused by pathogenic variants in the SERPING1 gene.

(3) HAE with normal C1-INH (nl-C1INH-HAE): This is a rare subtype of HAE (<5%) wherein the levels and functional activity of C1-INH protein is normal. nl-C1INH-HAE may be caused by mutations in FXII gene, ANGPTI *(Angiopoietin-1) gene, plasminogen gene, kininogen gene, myoferlin gene* and *heparan sulfate 3-O- sulfotransferase 6 gene*.[5]

to have symptoms in the childhood, pediatricians have a crucial role in early diagnosis of this disease.

In this review, we discuss the clinical manifestations, pathogenesis, diagnosis and management of HAE.

Classification of HAE (Box 2)

The basic abnormality in HAE patients i.e., deficiency of C1 inhibitor (C1-INH) protein was identified more than 60 years ago. C1-INH is a protease inhibitor in the serpin super family.[4] HAE is divided into various subtypes based on the level and functional activity of C1-INH protein.[5]

Pathophysiology of HAE

Bradykinin is the main cause of swelling in patients with HAE.[6] Bradykinin is a product of plasma contact system activation [comprises of factor XII, pre-kallikrein and high-molecular-weight-kininogen (HMWK)]. Bradykinin is generated when plasma kallikrein cleaves HMWK[7] (Fig.1). C1-INH is a serine protease inhibitor and mainly synthesized in hepatocytes.[8] It inhibits multiple proteases involved in the complement system, contact-system, coagulation and fibrinolytic pathway.[9] HAE type 1 and 2 are a result of a pathogenic variant in the *SERPING1* (Serpin family G member) gene that encodes for C1-INH.[10] A de novo mutation (where there is no suggestive family history) of SERPING1gene may be encountered in 20-25% of all patients.[11, 12]

In plasma contact system, kallikrein is generated from inactivated pre-kallikrein by protease factor XII that gets auto activated on exposure to negatively charged surface. C1-INH inhibits both factor XII and plasma kallikrein (Fig.1). Therefore, deficiency of C1-INH will lead to uncontrolled activation of factor XII and kallikrein causing excessive bradykinin formation (Fig.1) and hence increased vascular permeability through bradykinin B2 receptor.[13] Exaggerated bradykinin signalling is also responsible for swelling episodes in patients with nl-C1INH-HAE.

Clinical features

Majority of the patients with HAE-1 and 2 have onset in the childhood. Mean age of symptom onset has been noted to be between 8-12 years.[14] It may rarely present in infancy or during adulthood. Even though positive family history is an important clue for diagnosis, it may not be present in up to 25% of all patients.[12]

The interval of angioedema attacks varies from one patient to another.[15] Most attacks are spontaneous.

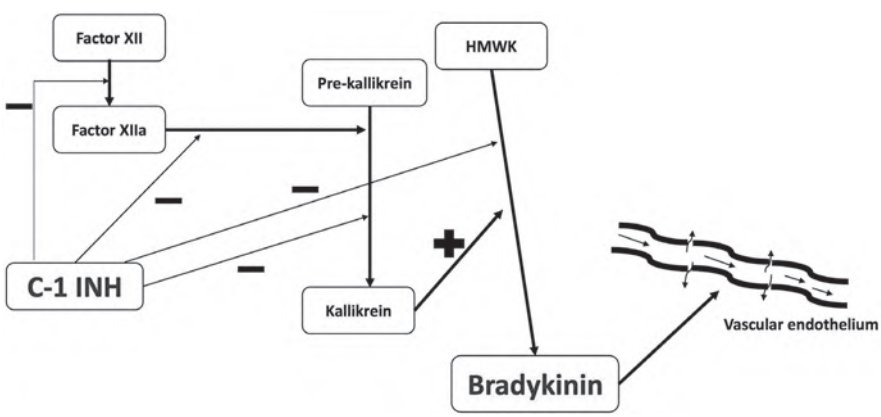


Fig.1. Pathophysiology of HAE. Factor XII is activated through contact with negatively charged surface. Activated factor XII leads to activation of pre-kallikrein to kallikrein that converts high molecular weigh kininogen (HMWK) to bradykinin. Bradykinin acts on vascular endothelium and causes excess leakage of fluid. C1-INH regulates production of bradykinin at several points in this pathway.

However, the episodes may be triggered by stress, blunt trauma, medical or surgical (including dental) procedures, infections, menstruation, estrogen replacement therapy or use of angiotensin convertase enzyme inhibitors. Few patients report food and change in weather as a trigger for their symptoms. The triggers are quite variable and are often patient specific.

Prodromal symptoms may be appreciated in approximately half of all patients with HAE and are important clues to differentiate HAE from other causes of angioedema. These may be in the form of tightness, tingling, fatigue or erythema marginatum like skin lesions and may present few hours before an attack of angioedema.[16]

Recurrent episodes of non-itchy, non-pitting, asymmetric and disfiguring swelling involving face (lips and eyelids), extremities, abdomen and genitals is the most common presentation (Fig.2).[17] These swelling episodes do not respond to antihistamines as this is bradykinin mediated, nor do they respond to glucocorticoids and epinephrine. Severe abdominal pain may be a presenting clinical feature and develops because of submucosal edema in the bowel loops. Abdominal pain may be associated with anorexia, vomiting, diarrhoea or intestinal obstruction in severe cases. This may mimic an acute abdomen and may often lead to inadvertent surgical interventions.[16]

Laryngeal edema is the most severe complication seen in HAE. All patients are at risk of of laryngeal involvement and more than half experience at least one attack of laryngeal edema during their lifetime.[18] Initial manifestation is in the form of buccal or lingual edema, voice changes or tightness at throat that may ultimately lead to difficulty in swallowing or stridor within few minutes. In the past, the mortality due to laryngeal attacks in HAE patients used to be approximately 30%.[16]

Patients with nl-C1INH-HAE present with a similar phenotype. However, prodromal manifestations such as erythema marginatum are generally absent and age of presentation is relatively as compared to HAE-1 and 2.[10,14]

Rare clinical manifestations of HAE include CNS involvement (presenting as seizure or stroke), pancreatitis, bladder, muscle and joint swelling.[14]

Laboratory diagnosis

In all patients with suspicion of HAE, C4, C1-INH levels and C1-INH function should be assessed. C1-INH levels are measured using nephelometry while C1-INH function is usually assessed using enzyme linked immunosorbent assay (ELISA). A simplified algorithm for diagnosis of various types of HAE is given in Fig.3.[5] Isolated measurement of C4 levels for screening is although cost effective, it has a 75-80% sensitivity and approximately 20-25% patients may have normal levels of C4 even at the time of attacks.[19,20] Repeat testing may be required in few patients who have normal results but have a strong clinical suspicion (this may preferably be carried out at the time of attacks).

C1q levels should be measured to differentiate HAE from acquired causes of bradykinin mediated angioedema such as those seen in autoimmune diseases.[16] C1q is always normal in patients with HAE, while it may be low in patients with autoimmune diseases.

Serpin family G member 1 (SERPING1) gene sequencing is recommended in all patients if available.[10,11,21]

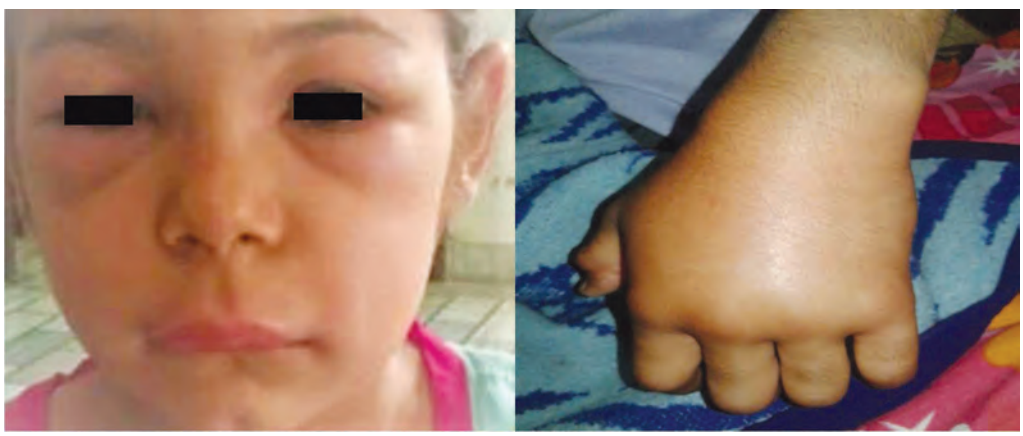


Fig.2. Lips and eyelids in a 6-year-old girl with HAE (left panel) and swelling of hands in a 15-year-old boy with in HAE (right panel)

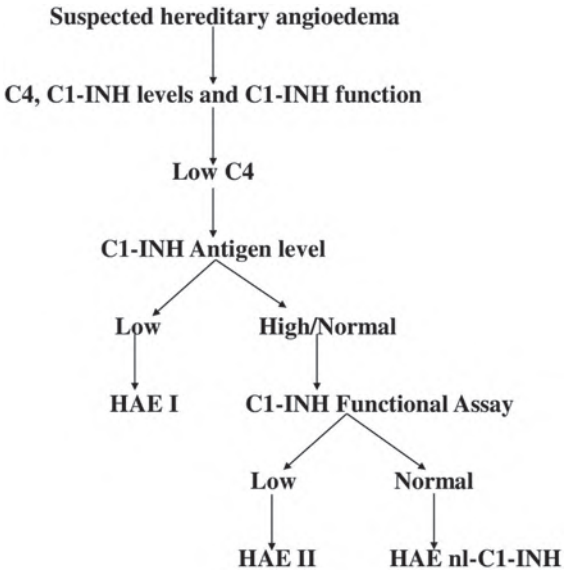


Fig.3. Simplified algorithm for laboratory diagnosis of a suspected case of HAE. C1q may be assayed in patients with suspected acquired angioedema (delayed onset of disease and no family history).

In patients with nl-C1INH-HAE, both C4 and C1-INH levels are normal (Table I) and genetic testing is required to diagnose these cases.[10,11]

Management

As HAE is a genetic disease, there is no permanent cure for this disease. Aim of treatment is to improve quality of life of patients, so that they have no or minimal episodes of angioedema and to prevent any disease related mortality. Treatment of HAE may be categorized into termination of acute episodes (on-demand treatment), long term prophylaxis (for prevention of attacks) and short-term prophylaxis. (to be given in situations where an attack is expected to occur such as during the dental procedures).

On-demand treatment (Table II) - Most international guidelines suggest that all acute episodes should be treated.[5,22,23] These attacks are treated using C1-INH concentrate, icatibant or ecallantide., a synthetic protein and a plasma kallikrein inhibitor, Tranexamic acid or androgens are not effective for treatment of acute attacks.[5] The advantages of on-demand treatment are early termination of attack and reduction of disease associated morbidity.[24] Every patient should be prepared for treatment of acute attacks by having stock of self-administered medications, because attacks may recur despite long-term prophylaxis.[5,22]

C1-INH concentrate can either be available in plasma derived form or recombinant form. Replaced C1-INH protein inhibits contact system and decreases production of bradykinin. Efficacy and tolerance are same for both plasma derived and recombinant forms.[25] C1-INH concentrate is administered through intravenous route for on-demand therapy. However, icatibant (a bradykinin B2 receptor inhibitor)[26] and ecallantide (a kallikrein inhibitor, licensed only for use in the USA) can be administered through subcutaneous route for on-demand therapy.[27] Ecallantide should be administered under supervision of health care provider because of chances of anaphylactoid reactions, while others can be self-administered.

In India, C1-INH therapy is not available at present.[28] Therefore, all life-threatening episodes of angioedema are managed using fresh-frozen-plasma (FFP).[21,28] FFP contains C1-INH in a concentration of 1 unit/ml. Dose of FFP is 10-20 ml/kg (maximum 2 units).

Table I. Molecular aspects and C4 & C1-INH status in various types of HAE

Types	Molecular defect	C4 Level	C1-INH levels	C1-INH Function
HAE-1	Serpin Family G Member 1(SERPING1) gene mutation leading to low C1-INH levels	Low	Low	Low
HAE-2	SERPING1 gene mutation leading to normal C1-INH levels but functionally defective C1-INH protein	Low	Normal	Low
HAE with normal C1 INH (nl-C1INH-HAE)	Mutations in FXII gene, ANGPTI (Angiopoietin-1) gene, Plasminogen gene, Kininogen gene and heparan sulfate 3-O-sulfotransferase 6 gene	Normal	Normal	Normal

Table II. Summary of drugs used for management of acute attacks of HAE

Drug	Regulatory status	Self-administration	Dosage	Mechanism of action	Side effects
Plasma derived C1-INH	Licensed for use in adolescents and adults of more than 12 years in USA and for patients of all ages in Europe	Yes	20U/kg intravenous	Inhibition of plasma kallikrein and coagulation factor XIIa	Chances of anaphylaxis, transmission of infectious agents
Ecallantide	Licensed for use inpatients of more than 16 years in USA	No	30 mg subcutaneous	Inhibition of plasma kallikrein	Uncommon-anti drug antibodies, risk of anaphylaxis
Icatibant	Licensed for use in USA and Europe for patients of more than 18 years	Yes	30 mg subcutaneous	Antagonism of bradykinin B2 receptor	Common - injection site reactions
Recombinant human C1-INH	Licensed for use in adults in Europe	No	50U/kg or 4200 U (whichever is higher) intravenous	Inhibition of plasma kallikrein and coagulation factor XIIa	Uncommon-risk of anaphylaxis
Fresh frozen plasma	Not approved	No	10-20 ml/kg or 2 units	Replaces C1-INH protein	Infusion reactions, transmission of viral agents, volume overload

The attack may begin to show improvement in 90 minutes to 12 hours in most patients.[29] There is a theoretical risk of aggravation of acute attacks with use of FFP as it also contains kininogen that may lead to increase in bradykinin production.[16] There are risks of transfusion transmitted infections, febrile reactions and volume overload with use of FFP.

(1) Prophylactic treatment

(a) Short-term prophylaxis - Short term prophylaxis may be required before any surgical or dental procedures or invasive medical interventions (such as endoscopy) as these procedures may trigger an acute attack.[22] C1-INH concentrate is an effective treatment option for short term prophylaxis.[30] However, in resource constrained settings where C1-INH is not available, attenuated androgens and FFP may be used for short term prophylaxis.[21,28,31] FFP is administered at a dose of 2 units or 10ml/kg 1 to 12 hours before procedure.[31] As risk of developing angioedema is very high in the first 24-48 hours after the procedure, dose of FFP may be repeated during this time. Attenuated androgens may be used for short-term prophylaxis [2-4 mg/day of stanozolol or 100-600 mg/day of danazol]. This should be initiated 2 days prior to the procedure and to be continued for 5 days after the procedure. If someone is already taking danazol or stanozolol as long-term prophylaxis, then dose may be doubled, 2 days before the procedure and should be continued for 5 days after the procedure.

(b) Long-term prophylaxis (Table III) - There are no strict guidelines for indications of long-term prophylaxis. However, it may be reasonable to start long-term prophylaxis in patients who experience at least more than one episode of angioedema every month or who has life threatening laryngeal attacks.[32]

Commonly used agent for prophylaxis is plasma-derived C1-INH. Although intravenous plasma-derived C1-INH (Cinryze) prophylaxis was the only option until recently, [33] subcutaneous C1-INH (Haegarda) is now available with good safety profile and excellent efficacy, and with advantage of subcutaneous administration.[34]

In developing countries where C1-INH is not available, attenuated androgens with submaximal androgenic properties such as danazol and stanozolol may be used as an alternative. They are efficacious and can be administered orally, but one should be cautious of several

Table III. Summary of drugs used for long-term prophylaxis

Drug	Regulatory Status	Dosage	Mechanism of action
Plasma derived C1 INH	Licensed in United States and Europe for patients ≥12 years	1000 U intravenous every 3-4 days	Inhibits plasma kallikrein, coagulation factor XIIa
Lanadelumab*	Licensed for long-term prophylaxis by FDA for patients aged 12 years or older in 2017	300 mg every 2 weekly	Fully humanized IgG1 monoclonal antibody directed against plasma kallikrein
Berotralstat#	FDA approved for prophylaxis in adults and children more than 12 years old	150 mg OD with food	Selective kallikrein inhibitor
Danazol	Approved in United States for adults	100 mg alternate days to 600 mg/day	17-α alkylated androgen, acts as an inducer of C1-INH synthesis in liver, causes more expression of C1-INH mRNA in mononuclear cells in the peripheral blood, increases catabolism of bradykinin by inducing aminopeptidase P activity
Stanozolol	Approved in United States for adults and children	0.5mg alternate days to 4 mg/day	17-α alkylated androgen, acts as an inducer of C1-INH synthesis in liver, increases catabolism of bradykinin by inducing aminopeptidase P activity
Tranexamic acid	Not approved for HAE	30–50 mg/kg/day in 2-3 divided doses (maximum dose3 g/day)	Antifibrinolytic, acts as a competitive inhibitor of plasminogen, reduces conversion of plasminogen to plasmin, prevents plasminogen mediated activation of FXII

**Common side effects are mild injection site reactions, dizziness, prolonged APTT and rare risk of anaphylaxis*

#Common side effects are abdominal pain, vomiting, diarrhea, back pain and heart burn

Table IV. An overview of various side effects of attenuated androgens

Androgenic effects	virilization precocious puberty amenorrhea acne infertility aggressive behavior and depression
Anabolic effects	accelerated or discontinued growth hypertension and headache weight gain muscle cramps polycythemia dyslipidemia

adverse effects associated with prolonged use of these drugs[28, 35] (Table IV). Therefore, these drugs should be used in minimal effective dose.

In resource constraint setting, tranexamic acid is also an option for long term prophylaxis.[28] Although it has fewer side effects as compared to attenuated androgens, it is less effective than attenuated androgens.[5] However, because of higher risk of side effects with use of androgens in children and pubertal age group (androgens are not recommended for use in children by most international guidelines), tranexamic acid is frequently used as long-term prophylaxis in children with HAE in India.

Conclusion

HAE is an uncommon disease and presents with a variable clinical presentation. Because of lack of

awareness, most patients remain undiagnosed and untreated. C4, C1-INH levels and C1-INH functional assay should be carried out in all patients with suspected HAE. Most of the first line treatments for HAE are not available in India. Hence, FFP remains the drug of choice for acute treatment and short-term prophylaxis. Attenuated androgens (stanozolol and danazol) can be used for long-term prophylaxis and short-term prophylaxis while tranexamic acid is used for long-term prophylaxis especially in children.

Points to Remember

- ***Hereditary angioedema (HAE) is an uncommon disorder characterized by episodic edema.***
- ***Because of lack of awareness, the disease remains undiagnosed for several years.***
- ***HAE should be suspected in all patients who present with episodic edema without urticaria.***
- ***In patients with suspected HAE, C4, C1-INH levels and C1-INH function should be assessed.***
- ***Most patients have diseases onset in childhood. Hence, pediatricians have an important role to play in the early diagnosis of HAE.***
- ***Patients with HAE in most of the developing countries including India are managed using fresh frozen plasma, attenuated androgens and tranexamic acid because all 1st line treatments are not available.***

References

1. Nussberger J, Cugno M, Amstutz C, Cicardi M, Pellacani A, Agostoni A. Plasma bradykinin in angio-oedema. Lancet 1998; 351:1693-1697.
2. Bork K, Hardt J, Witzke G. Fatal laryngeal attacks and mortality in hereditary angioedema due to C1-INH deficiency. J Allergy Clin Immunol 2012; 130(3):692-697.
3. Reshef A, Kidon M, Leibovich I. The story of angioedema: from Quincke to bradykinin. Clin Rev Allergy Immunol 2016; 51:121-39.
4. Donaldson VH, Evans RR. A biochemical abnormality in hereditary angioneurotic edema: absence of serum inhibitor of C2 1-esterase. Am J Med 1963; 35:37-44.
5. Maurer M, Magerl M, Ansotegui I, Aygören-Pürsün E, BetschelS, Bork K, et al. The international WAO/EAACI guideline for the management of hereditary angioedema-the 2017 revision and update. Allergy 2018; 73:1575-1596.
6. Schmaier AH. The contact activation and kallikrein/kinin systems: pathophysiologic and physiologic activities. J Thromb Haemost 2016; 14(1):28-39. doi: 10.1111/jth.13194. Epub 2016 Jan 11. PMID: 26565070.
7. Kaplan AP, Joseph K. Pathogenesis of hereditary angioedema: the role of the bradykinin-forming cascade. Immunol Allergy Clin North Am 2017; 37: 513-525.
8. Frank MM. Complement disorders and hereditary angioedema. J Allergy Clin Immunol 2010; 125:S262-S271.
9. Kaplan AP, Joseph K. Complement, kinins, and hereditary angioedema: mechanisms of plasma instability when C1 inhibitor is absent. Clin Rev Allergy Immunol 2016; 51:207-215.
10. Sharma J, Jindal AK, Banday AZ, Kaur A, Rawat A, Singh S, et al. Pathophysiology of Hereditary Angioedema (HAE) Beyond the SERPING1 Gene. Clin Rev Allergy Immunol 2021 Jan 14. doi: 10.1007/s12016-021-08835-8. Epub ahead of print. Erratum in: Clin Rev Allergy Immunol 2021; PMID: 33442779.
11. Banday AZ, Kaur A, Jindal AK, Rawat A, Singh S. An update on the genetics and pathogenesis of hereditary angioedema. Genes Dis 2019; 7(1):75-83. doi: 10.1016/j.gendis.2019.07.002. PMID: 32181278; PMCID: PMC7063419.
12. Pappalardo E, Cicardi M, Duponchel C, Carugati A, Choquet S, Agostoni A, et al. Frequent de novo mutations and exon deletions in the C1 inhibitor gene of patients with angioedema. J Allergy Clin Immunol 2000; 106:1147-1154.
13. Jacques L, Couture R, Drapeau G, Regoli D. Capillary permeability induced by intravenous neurokinins. Receptor characterization and mechanism of action. Naunyn Schmiedebergs Arch Pharmacol 1989; 340:170-179.
14. Busse PJ, Christiansen SC. Hereditary Angioedema. N Engl J Med 2020; 382(12):1136-1148. doi: 10.1056/NEJMra1808012. PMID: 32187470.
15. Saltoun C. Urticaria, angioedema, and hereditary angioedema. In: Grammer LC, Greenberger PA, eds. Patterson's allergic diseases. 8th ed. Philadelphia: Wolters Kluwer, 2018; pp689-708.
16. Zuraw BL, Bernstein JA, Lang DM, Craig T, Dreyfus D, Hsieh F, et al. A focused parameter update: hereditary angioedema, acquired C1 inhibitor deficiency and angiotensin-converting enzyme inhibitor-associated angioedema. J Allergy Clin Immunol 2013; 131:1491-1493.
17. Khan DA. Hereditary angioedema: historical aspects, classification, pathophysiology, clinical presentation and laboratory diagnosis. Allergy Asthma Proc 2011; 32: 1-10.
18. Bork K, Hardt J, Witzke G. Fatal laryngeal attacks and mortality in hereditary angioedema due to C1-INH deficiency. J Allergy Clin Immunol 2012; 130: 692-697.
19. Aabom A, Bygum A, Koch C. Complement factor C4 activation in patients with hereditary angioedema. Clin Biochem 2017; 50:816-821.

20. Zuraw BL, Christiansen SC. Hereditary angioedema and bradykinin-mediated angioedema. In: Adkinson NF Jr, Bochner BS, Burks AW, Busse WW, Holgate ST, Lemanske RF, O'Hehir R, editors. Middleton's allergy: principles and practice. 8th edn. Philadelphia: Elsevier Saunders, 2014; pp588-600.
21. Jindal AK, Rawat A, Kaur A, Sharma D, Suri D, Gupta A, et al. Novel SERPING1 gene mutations and clinical experience of type 1 hereditary angioedema from North India. Pediatr Allergy Immunol 2021; 32(3):599-611. doi: 10.1111/pai.13420. Epub 2020 Dec 5. PMID: 33220126.
22. Betschel S, Badiou J, Binkley K, Borici-Mazi R, Hébert J, Kanani A, et al. The International/Canadian Hereditary Angioedema Guideline. Allergy Asthma Clin Immunol 2019 Nov 25; 15:72. doi: 10.1186/s13223-019-0376-8. Erratum in: Allergy Asthma Clin Immunol 2020; 16:33. PMID: 31788005; PMCID: PMC6878678.
23. Busse PJ, Christiansen SC, Riedl MA, Banerji A, Bernstein JA, Castaldo AJ, et al. US HAEA Medical Advisory Board 2020 Guidelines for the Management of Hereditary Angioedema. J Allergy Clin Immunol Pract 2021; 9(1):132-150.e3. doi: 10.1016/j.jaip. 2020.08. 046. Epub 2020 Sep 6. PMID: 32898710.
24. Bork K, Staubach P, Hardt J. Treatment of skin swellings with C1-inhibitor concentrate in patients with hereditary angio-oedema. Allergy 2008 Jun; 63(6):751-757. doi:10.1111/j.1398-9995.2007.01577.x. PMID:18445189.
25. Craig TJ, Levy RJ, Wasserman RL, Bewtra AK, Hurewitz D, Obtu³owicz K, et al. Efficacy of human C1 esterase inhibitor concentrate compared with placebo in acute hereditary angioedema attacks. J Allergy Clin Immunol 2009; 124(4):801-808. doi: 10.1016/j.jaci. 2009.07.017. Epub 2009 Sep 19. PMID: 19767078.
26. Lumry WR, Li HH, Levy RJ, Potter PC, Farkas H, Moldovan D, et al. Randomized placebo-controlled trial of the bradykinin B_2 receptor antagonist icatibant for the treatment of acute attacks of hereditary angioedema: the FAST-3 trial. Ann Allergy Asthma Immunol 2011; 107(6):529-537. doi: 10.1016/j.anai.2011.08.015. Epub 2011 Oct 5. PMID: 22123383.
27. Cicardi M, Levy RJ, McNeil DL, Li HH, Sheffer AL, Campion M, et al. Ecallantide for the treatment of acute attacks in hereditary angioedema. N Engl J Med 2010; 363(6):523-531.
28. Jindal AK, Reshef A, Longhurst H, GEHM workgroup (Global Equity in HAE Management). Mitigating Disparity in Health-care Resources Between Countries for Management of Hereditary Angioedema. Clin Rev Allergy Immunol 2021. doi: 10.1007/s12016-021-08854-5. Epub ahead of print. PMID: 34003432.
29. Prematta M, Gibbs JG, Pratt EL, Stoughton TR, Craig TJ. Fresh frozen plasma for the treatment of hereditary angioedema. Ann Allergy Asthma Immunol 2007; 98(4):383-388. doi: 10.1016/S1081-1206(10)60886-1. PMID: 17458436.
30. Bork K, Hardt J, Staubach-Renz P, Witzke G. Risk of laryngeal edema and facial swellings after tooth extraction in patients with hereditary angioedema with and without prophylaxis with C1 inhibitor concentrate: a retrospective study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112(1):58-64.
31. Jindal AK, Singh A, Anjani G, Kaur A, Jaiswal M, Chopra S, et al. Successful perioperative management of three patients with hereditary angioedema without C1 esterase inhibitor therapy: A developing country perspective. Immunobiology 2020; 225(6):152022. doi: 10.1016/j.imbio.2020.152022. Epub 2020 Nov 3. PMID: 33197705.
32. Longhurst H, Zinser E. Prophylactic Therapy for Hereditary Angioedema. Immunol Allergy Clin North Am. 2017; 37(3):557-570. doi: 10.1016/j.iac.2017.04.003. PMID: 28687109.
33. Zuraw BL, Busse PJ, White M, Jacobs J, Lumry W, Baker J, et al. Nanofiltered C1 inhibitor concentrate for treatment of hereditary angioedema. N Engl J Med 2010; 363(6):513-522.
34. Longhurst H, Cicardi M, Craig T, Bork K, Grattan C, Baker J, et al. Prevention of Hereditary Angioedema Attacks with a Subcutaneous C1 Inhibitor. N Engl J Med 2017; 376(12):1131-1140. doi: 10.1056/NEJMoa 1613627. PMID: 28328347.
35. Bork K, Bygum A, Hardt J. Benefits and risks of danazol in hereditary angioedema: a long-term survey of 118 patients. Ann Allergy Asthma Immunol 2008; 100(2):153-161. doi: 10.1016/S1081-1206(10)60424-3. PMID: 18320917.

GLOSSARY IN IMMUNOLOGY

NETosis: In addition to the well-established mechanisms, neutrophils also exhibit 'NETosis', the process of neutrophil extracellular trap (NET) formation helps in trapping and killing any infectious agent. NETs are extracellular webs of chromatin, microbicidal proteins, and oxidant enzymes that are released by neutrophils to contain infections. Although, this recently discovered function of neutrophil is debatable it has gained essential research. NET generation is suspected to play a role in severe COVID-19. Research into medications that display NETs formation regulatory properties as potential significant therapeutic strategies in the progress of COVID-19.

GENERAL ARTICLE

EARLY BEHAVIORAL SIGNS OF ATTENTION DEFICIT HYPERACTIVITY DISORDER: A PRACTICAL GUIDE

***Vijaya Raman**

Abstract: *Attention deficit hyperactivity disorder is one of the most common neurobehavioral disorders seen in childhood. It is important to keep a high index of suspicion when parents complain of behavioral issues in young children. Many behavioral changes in early childhood do cease to be of concern when children grow up. There are some definite early indicators of behavioral issues that continue to be problematic and affect the development and later functioning in all areas. This article focuses on early identification of problem behaviors that may lead to negative short and long-term effects on an individual's personal and professional life.*

Keywords: *Early identification, Behavior, Children.*

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by hyperactivity, impulsivity and inattentive behaviour. Not all children diagnosed with ADHD will have all these three dimensions. Some children are predominantly hyperactive and impulsive, while others are mainly inattentive. It affects about 5-11% of children worldwide including India.[1,2] It is more commonly seen among boys than girls (about 3:1).[3]

The mean age at diagnosis of ADHD is around seven years. Usually by the time a child with ADHD reaches this age, parents are already aware that their child's inattentiveness, level of activity, or impulsiveness are greater than a typical child. However, if the condition is diagnosed even before the child enters school, then there is an opportunity to change the developmental and behavioral trajectory of these children by early intervention strategies at home and at pre-school.[4] It is important to consider the fact that children who are diagnosed later, have more dysfunction and disability associated with ADHD, including difficulties in learning, social functioning and self- regulation. This leads to reckless behavior, low self-esteem and lack of confidence due to the fallout of their behaviors. Longitudinal studies show that two-thirds of children with ADHD will continue to have symptoms in adulthood.[5]

A well informed parent and the observant primary care pediatrician are in a good position to enable the early diagnosis of ADHD. It is, therefore, imperative that some practical guidelines are made available for their routine use in practice.

Practice guidelines have been published by the various academies of pediatrics including the Indian Academy of Pediatrics.[6] All of them, however, focus on the school going children - 6 years and above.

The question to be asked is - 'Are there any early behavioral indicators that might help to identify children at future risk for ADHD?' This article attempts to answer this question.

Temperamental precursors to ADHD

Temperament is defined as the behavioral style that individuals consistently exhibit in reaction to their environments.[7] This is biologically driven and genetically linked which is evident early in life and is relatively stable over time and all situations.

ADHD is theorized to have temperamental precursors early in life. McIntosh and Cole-Love reported that children diagnosed with ADHD exhibited temperaments that is high in activity, high in distractibility and low in persistence on tasks.[8]

Foley, McClowry and Costellanos stated that symptoms of ADHD are highly correlated with dimensions of childhood temperament.[9] All three subtypes of ADHD - hyperactive - impulsive, inattentive and combined (exhibiting symptoms of both inattention and hyperactivity/ impulsivity) - were strongly associated with the temperamental dimensions of high negative reactivity, low task persistence, high activity, low attentional focusing, high impulsivity and low inhibitory control.

* Professor of Clinical Psychology,
Department of Psychiatry,
St. John's Medical College,
Bangalore.
email: vijaya.r@stjohns.in

In a study that examined a set of conceptually relevant emotional temperament indicators in 6 month old infants with a parental history of ADHD, the authors found that the most notable domain was infant anger/ irritability.[10]

In summary, research indicates that children who are diagnosed with ADHD in later life have some temperamental vulnerabilities as early as the first 2 years of life which include increased activity levels, difficulty in focussing on a task, impulsivity (acting without thinking of the consequences), easy distractibility, irritability, anger, stubborn behavior, tantrums, excessive crying and being difficult to pacify. Sometimes, these children may also have difficulty with biological rhythms like sleeping, feeding and toileting. It is, however, important to keep in mind that it is a problem only when these behaviors are persistent and present almost every day.

Family history

ADHD is usually heritable, with estimates well above 0.70.[11] (a heritability of 0.7 indicates that the phenotypic variation is 70% due to genetic variation). The disorder is also highly familial, with substantially increased risk in offspring of parents with ADHD, thus making parental ADHD an excellent proxy for offspring liability.

A study of 894 ADHD probands and 1135 of their siblings aged 5-17 years found a nine-fold increased risk of ADHD in siblings of ADHD probands compared to siblings of controls.[12] Adoption studies suggest that the familial factors of ADHD are attributable to genetic factors rather than shared environmental factors. Familial transmission is likely to be a complex interaction between environmental, genetic and epigenetic factors that contribute to the heterogeneity in ADHD symptomatology.

In summary, ADHD runs in families. It would be important, therefore, that if parents report behavioral issues in their child which is not manageable, it would be pertinent to ask if either of the parents had similar issues - both when they were children and also currently as adults. Poor parenting is not thought to cause ADHD, although poor parenting skills and a chaotic lifestyle may exacerbate symptoms in a child with the condition.

Early behavioral indicators

Usually, the diagnosis of ADHD is made between the ages of 6 and 12 years. It is more difficult to diagnose ADHD in children 5 years of age and younger. This is because children change very rapidly during the preschool years and many behaviors are normal or typical and children do grow out of them. However, if a child has comorbid medical conditions like prematurity, low birth weight, seizures or other forms of brain damage, features suggestive of ADHD may manifest even earlier.

Arnett, et al found out that ADHD symptoms have an onset prior to school age, and that ADHD could potentially be diagnosed in infancy or toddlerhood with better screening tools that would include behavioral and cognitive measures.[4] Appropriate early screeners for ADHD risk would measure externalizing and internalizing symptoms, sleep difficulties, social problems, cognitive performance and physiological measures of behavioral and attention regulation.

For the primary care pediatrician, some early behaviors that may need to be enquired into and recorded for follow up would need to be specified. The ability to discern is a skill every professional working with young children should develop when behavior is out of keeping

Table I. Referral guidelines for possible ADHD

Refer when the behavior…	Wait and watch when the behavior….
..has been observed for at least 6 months	..is recent and inconsistent
..is a problem in several settings	..appears at a single place or time
..occurs during independent and group activities	..occurs primarily when in group and during prolonged sitting
..cannot be explained by other circumstances or disabilities	..could be the result of recent life events
..interferes with learning	..when child is acquiring age appropriate skills
..affects peer relationships and social development	..demonstrates appropriate friendships and interactions
..is inappropriate despite clear, consistent age appropriate expectations	..varies in the presence of different adults in the child's life
..appears out of the child's control	..appears purposeful or attention seeking

with expected levels. When a child demonstrates a persistent inability to sustain attention, inability to respond with some thinking of consequences of an action and not having control over their activity, it would be wise to monitor. The fact remains that there is no test to diagnose ADHD and it is based almost on clinical judgement. It may be necessary, therefore, to document behaviors of concern over time and in different situations to enable an accurate diagnosis.

Fewell, et al have given some referral guidelines for possible ADHD (Table I).[13]

To summarize, obviously, some of these behaviors will be evident even in typically developing children when they are unwell or are overwhelmed by the clinical atmosphere and with increased anxiety. This behavior in typical children usually settles down after being comforted by the parent or the doctor or after some time when they get adjusted to the place. However, it can be considered as a problem if the behavior is persistent, disruptive and difficult to control.

The important point to remember is that if the behavior is not causing distress, dysfunction (if it is not coming in the way of the child's functioning in various domains like in preschool, at home, with peers etc.) and is not interfering with the child's cognitive, social or emotional development, then it is not a cause for immediate concern.

It is important to reiterate that many of these early behavioral concerns are more likely to be brought to the notice of the primary care pediatrician and it is also inappropriate to reassure without looking into these behaviors. A poster with the list of concerns can be displayed in the waiting room of the clinic to help parents to recognise those concerns which can be discussed with the pediatrician. There are also some resources available online to help give simple tips for parents that can also be shared in the waiting room. This would facilitate discussion, early identification, early intervention and prevent or at least reduce dysfunction and distress of the child and the family.

Overlap between autism spectrum disorder (ASD) and ADHD

Although there are some important differences (e.g. core symptom definition and recommended treatment), ASD and ADHD share many similar impairments in different domains that could complicate a differential diagnosis.[14] Clinically, there would be three domains that may help to differentiate behaviors of ASD and ADHD:

1. Attention span: While children with ASD and ADHD can have attention issues, the important difference would be that children with ADHD have a hard time paying attention to and sustaining attention on any task and get distracted while children with ASD will be able to sustain attention (sometimes for very long duration) on tasks that are of interest to them but not on others that they may not be interested.

2. Communication: Although communication difficulties are seen in children with ADHD as well, the differentiating factors would be in the intent to communicate (children with ASD may lack intent while children with ADHD do not), children with ADHD will interrupt others, have difficulty waiting for their turn and talk a lot. Children with ASD may struggle to use words, not use gestures, may fixate on a particular topic and not initiate or respond to social interactions.

3. Routine and structure: It is commonly seen that children with ADHD can become bored quickly with a structure that they find uninteresting and without variety, they may also lose interest in activities. In contrast, autistic children often demonstrate an insistence on sameness, adherence to routines or ritualized patterns of verbal or nonverbal behavior. A change in the routine can make them upset and irritable.

When and how to refer

It might not be possible for a pediatrician in a busy OPD to conduct detailed evaluation of a child with behavioral concerns If the parents repeatedly bring behavioral concerns to the notice of the pediatrician during routine clinic visits, it is ideal to refer rather than reassure.

It is important, therefore, that the pediatrician creates a network that includes psychologists, psychiatrists, developmental pediatricians, social workers and nurses who are trained in child development to facilitate referral and to enable follow up. Specific contact details would be helpful.

It is also important to note that some parents' threshold for complaining about their child's behaviour is lower than others. It will be helpful, in these, circumstances, to corroborate with the other parent or any other caregiver.

Conclusion

It is amply evident, on reviewing literature about early behavioral indicators of ADHD, that most children who are diagnosed with ADHD in later life, do display early behavioral signs that go unnoticed. The importance of early diagnosis and intervention cannot be overemphasized as,

like with other developmental disorders in children, early identification is the key to reduce dysfunction later.

The importance of developmental monitoring using available standardized tools, acknowledging parent/ caregiver concerns, documenting behaviors and maintaining dialogue with families need emphasis in practice. Early signs and symptoms, if made available to the primary care pediatrician, will enhance their ability to identify the problem, make an early diagnosis, counsel parents and provide management options.

Points to Remember

- ***ADHD is a common neurobehavioral disorder which is often undiagnosed till significant impairment is observed.***
- ***Repeated parental concerns regarding behavior of the child during routine OPD visits should warrant referral rather than reassurance.***
- ***There are early behaviors that can aid in early identification and intervention.***
- ***Early intervention prevents negative impact on the child's development, self-esteem and overall functioning and outcome.***

References

1. Polanczyk G, Silva de Lima M, Lessa Horta B, Biederman J, Rohde LA. The Worldwide Prevalence of ADHD: A Systematic Review and Meta-regression Analysis. Am J Psychiatry 2007; 164:942-948.
2. Suthar N, Garg N, Verma KK, Singhal A, Singh H, Baniya G. Prevalence of Attention Deficit Hyperactivity Disorder in primary school children: A cross - sectional study. J Indian Assoc. Child Adolesc. Mental Health 2018, 14(4):74-88.
3. Rucklidge JJ. Gender differences in attention deficit hyperactivity disorder. Psychiatr Clin North Am 2010; 33(2):357-373.
4. Arnett AB, MacDonald B, Pennington BF. Cognitive and behavioral indicators of ADHD symptoms prior to school age. J Child Psychol Psychiat 2013; 54(12):1284-1294.
5. Faraone SV, Larsson H. Genetics of attention deficit hyperactivity disorder. Molecular Psychiat 2019; 24: 562-575.
6. Dalwai S, Unni J, Kalra V, Singhi P, Shrivastava L, Nair MKC. Consensus statement of the Indian Academy of Pediatrics on evaluation and management of Attention Deficit Hyperactivity Disorder. Indian Pediatr 2017; 54:481-488.
7. Rothbart MK, Bates JE. Temperament. In: W. Damon (Series Ed.), N. Eisenberg (Vol. Ed.) Handbook of Child Psychology: Vol.3. Social, Emotional and Personality development, 6th edn. Hoboken, NJ: John Wiley & Sons, Inc 2012; pp105-176.
8. McIntosh DE, Cole- Love AS. Profile comparisons between ADHD and non-ADHD children on the temperament assessment battery for children. J Psychoeduc Assess 1996; 14:362-372.
9. Foley M, McClowry SG, Castellanos FX. The relationship between attention deficit hyperactivity disorder and child temperament. J Appl Devl Psychol 2008; 29:157-169.
10. Sullivan EL, Holton KF, Nousen EK, Barling AN, Sullivan CA, Propper CB, et al. Early identification of ADHD risk via infant temperament and emotion regulation: a pilot study. J Child Psychol Psychiat 2015; 56(9): 949-957.
11. Kan KJ, Dolan CV, Nivard MG, Middeldorp CM, van Beijsterveldt CE, Willemsen G, et al. Genetic and environmental stability in attention problems across the lifespan: Evidence from the Netherlands twin register. J Am Acad Child Adolesc Psychiatry 2013; 52:12-25.
12. Chen W, Zhou K, Sham P, Franke B, Kuntsi J, Campbell D, et al. DSM IV combined type ADHD shows familial association with sibling trait scores: a sampling strategy for QTL linkage. Am J Med Genet B Neuro Psychiatr Genet 2008; 147B:1450-1460.
13. Fewell RR, Deutscher B. Attention Deficit Hyperactivity Disorder in Very Young Children: Early signs and interventions. Inf Young Children 2002; 14(3):24-32.
14. Craig F, Lamanna AL, Margari F, Matera E, Simone M, Margari L. Overlap between Autism Spectrum Disorders and ADHD: Searching for Distinctive/ Common Clinical Features. Autism Res 2015; 8:328-337.

GLOSSARY IN IMMUNOLOGY

Next-generation sequencing (NGS): This is a massively parallel sequencing technology that offers ultra-high throughput, scalability and speed. Also known as high-throughput sequencing, is the catch-all term used to describe a number of different modern sequencing technologies. The technology is used to determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA. These technologies allow for sequencing of DNA and RNA much more quickly and cheaply than the previously used Sanger sequencing and as such revolutionised the study of genomics and molecular biology.

DRUG PROFILE

IRON CHELATION IN CHILDREN

***Jeeson C. Unni**

Abstract: *Currently, the goal of iron chelation has shifted from treating iron overload to preventing iron accumulation and iron-induced end-organ complications, in order to achieve a complication-free survival and an improved quality of life of children with iron overload. New chelation options increase the likelihood of achieving these goals. Timely initiation, close monitoring and continuous adjustment are the cornerstones of optimal chelation therapy in children. Despite use of iron chelators for more than 60 years, grey areas still remain. The three available iron chelators have been reviewed.*

Keywords: *Iron overload, Iron chelators, Desferrioxamine, Deferiprone, Deferasirox.*

Many congenital / acquired anemias and other conditions are associated with iron overload in childhood. The rate of iron deposition differs according to the source of excess iron (transfusional versus non-transfusional), the underlying condition and the type of iron chelation therapy. Iron accumulation is slow with non-transfusional loading but cumulative and can present with features of iron overload by around 10-15 years. The rate of iron deposition differs according to the source of excess by the second decade. Transfusional iron loading is rapid. On an average a unit of pRBC prepared from 420ml of blood has about 200 mg of iron.[1] Once transferrin (Tf) saturation occurs after 4-6 transfusions, harmful iron - non-transferrin bound iron (NTBI) and a specific portion of NTBI, the chelatable labile plasma iron (LPI), which is found in healthy individuals[2] - accumulates and starts getting deposited in tissues. LPI, the plasma iron component is a fraction of NTBI which is the earliest measurable parameter indicating iron overload. Repeated transfusions for 2 years or more would cause severe iron burden as estimated by serum ferritin (SF) and liver iron concentration (LIC).[3]

* Editor-in-chief,
IAP Drug Formulary,
Sr. Consultant, Dept. of Child and Adolescent Health,
Aster Medcity,
Kochi, Kerala.
email: jeeson1955@gmail.com

Chelation therapy aims to balance the rate of iron accumulation in the body by increasing iron excretion in urine and or faeces. Careful dose adjustment is necessary to avoid excess chelation as iron levels fall. A major concern is compliance. Treatment has to be continued throughout life, as even short periods of discontinuation can have deleterious effects. Convenience of administration and tolerance of the medication along with child's acceptance and support of the family, the pediatrician and the institution are factors that determine adherence and outcome.

Pathophysiology of iron overload

The normal distribution of iron is depicted in Box 1.

Non transferrin bound iron (NTBI): It is the term used for the free iron present in plasma, which has the potential to generate free radicals oxidative stress to organs like heart, pancreas and RBCs. Normally iron is bound to transferrin or other iron binding proteins like haem, apoferritin, hemosiderin etc. NTBI is kept at negligible levels by transferrin and it is also regulated by hepcidin, a hormone produced in liver.

Iron homeostasis: Daily production of new RBCs requires 20-24 mg of iron. This is primarily obtained from macrophage-mediated catabolisation of hemoglobin from senescent RBCs, which is about 20 mg. Dietary iron absorption in the duodenum is 1-2 mg/day. Hence, iron homeostasis is composed of a closed loop from plasma transferrin to RBCs, from RBCs to macrophages and from macrophages to plasma transferrin. In normal physiological conditions, the level of transferrin is sufficient for complete scavenging of even the greater dose of free iron and ensuring its absence in internal milieu.

Box 1. Distribution of iron

- Total pool of iron in the human body is 3-3.5g
- Storage capacity is 7g
- RBC Hb - 60%
- Stored in cells as ferritin - 25%
- Myoglobin, heme and non-enzymes - 15%
- NTBI or plasma iron - <1 μmol/L (negligible amount or undetectable)

Iron overload due to transfusion: A 100 ml of PRBC delivers 108 mg of iron. When the iron load is higher because of multiple transfusions, transferrin is saturated, resulting in high levels of NTBI. There are no effective physiological methods of iron excretion. This is the reason why levels of NTBI in normal healthy individual do not exceed 1 μmol/L. After repeated transfusions, this safety mechanism is overwhelmed.

Iron chelators licensed for use in children

Till date, 3 iron chelators are licensed for clinical use. Although deferoxamine (desferrioxamine / DFO) has been the gold standard treatment for transfusional iron overload in the past four decades, compliance issues hamper its effectiveness (Table I).[4]

Deferasirox (DFX) has been approved as first line therapy for the treatment of transfusional iron overload in children more than 2 years of age. DFX was the treatment of choice for the majority of children and adolescents, until recently, due to non-availability of alternative oral chelators.[5,6] DFX is also used in children 10 years and older with non-transfusion-dependent thalassaemia syndromes

Deferiprone (DFP) has been licenced for the treatment of iron overload in patients with thalassemia major when DFO is contraindicated or inadequate.[7] Its use has been approved in the USA as second line therapy for patients with thalassaemia who have an inadequate response or contraindication to DFO or DFX. However, there is evidence that adverse events are increased in patients treated with DFP compared with DFO and in patients treated with combined DFP and DFO compared with DFO alone.

DFO is not tolerated well by many children as it has to be given as daily infusions at least 5 times a week. Limiting factors in the use of DFO and DFP are their toxicity and DFX, the most child friendly preparation of the three, is non-superior to DFO for chelating cardiac iron.

There is, therefore, a sustained interest among researchers to develop new iron chelator molecules. N, N-bis(2-hydroxybenzyl)ethylenediamine-N, N-diacetic acid (HBED), pyridoxal isonicotinoyl hydrazone (PIH), SPD-602 and CMI are novel oral iron chelators in clinical development.[8,9]

Current challenges in iron chelation therapy

i) Monotherapy is inadequate to ensure negative iron balance. Although there are 3 iron chelators (Table I) used for the treatment of iron overload, often treatment with any of these chelators alone is not sufficient and it is estimated that 20% of patients undergoing iron chelation therapy will be inadequately chelated.[10]

ii) Large amounts of iron accumulate during transfusion therapy- Excess iron may accumulate in liver and in organs that don't normally store iron, such as the pancreas, heart, joints, endocrine organs and skin leading to free radical damage in these areas. Since there are several iron pools that develop in iron overload, chelators which are effective at mobilizing iron from all labile iron pools would be advantageous, especially from the heart. The ability of present chelators to remove iron deposited in tissues is inadequate.[10]

iii) Inability to bind NTBI over long periods of time - A long-acting chelator would ultimately decrease the

Table I. Iron chelating agents

Name of the chelator	Average cost for a 10 kg child per month	Circulation $t_{1/2}$	Route of administration	Dose mg/kg /day	Efficacy for removal of stored iron	Specific side effects to be monitored
Desferrioxamine (DFO)	INR 4000	Short (~20 min) requires all-day (8-12 h) delivery	IV/SC	20-40	Liver/ Cardiac	Visual field defects, high frequency hearing loss, sitting height
Deferiprone (DFP)	INR 600	Moderate; requires at least 3 times per day dosing	Oral	75-100	Cardiac	Agranulocytosis, arthralgia
Deferasirox (DFX)	INR 1000	Ideal; 8–16 hours, requiring once-daily dosing	Oral	7-21	Liver	Rise in SGPT, creatinine, gastritis, dermatitis

amount of iron that is taken up into tissues and would prevent harmful, iron-catalyzed reactions. Irregular chelation with presently available chelators causes iron overload.

An ideal iron chelator should be affordable for patients in low-income countries, palatable for oral administration, have a circulation t½ that is long enough to allow once-daily dosing and effective iron removal, a high therapeutic index, have minimal toxicity and be efficient in removing iron from heart, liver, endocrine organs, etc. It must have high capacity to chelate unsaturated iron binding capacity that lasts long enough to prevent drastic fluctuations in LPI which is low with DFO, moderate with DFP and high with DFX.

Combination chelation

Combining two chelators either given simultaneously or sequentially has been studied over the years and has proven efficacy in decreasing iron overload. The simultaneous combination works on the "shuttle hypothesis"- whereby one chelator mobilizes cell iron and the other chelates the iron in plasma. DFO and DFP combination has been validated to reduce life-threatening iron deposition and those presenting with cardiac complications.[11] The combination of DFX and DFP has been shown to reduce liver and cardiac iron with tolerable toxicity and combined treatment with DFX and DFO is more effective than DFX for reduction of iron overload.[12]

Compliance to iron chelators in our country

Iron overload does not result in overt clinical symptoms. Hence, ensuring optimal chelation long term requires motivated children and adolescents and their families and caregivers. This is especially true of the injectable medications. The main issue in low and middle income countries is financial constraints. Adolescence is a particularly difficult time with mood swings, depression and pressure of academic commitments causing poor compliance. The transition from pediatric to adult services also needs to be factored.

Desferrioxamine

Desferrioxamine (DFO) is a trivalent iron chelator licensed for use in children above two years of age. It is administered either subcutaneously or via the intravenous route at a dose of 20-40 mg/kg/day. One vial of 500mg of DFO when diluted in 5ml of water for injection results in a 10% solution. The recommended dose is a slow subcutaneous infusion over 8-12 hours of a 10% solution with an infusion pump for a minimum of 5 days a week. It can be administered as bolus subcutaneous doses once or twice a day or as a continuous intravenous infusion through a balloon pump. In children, the mean daily amount should not exceed 40mg/kg/day. The half-life of DFO is extremely short and therefore its efficacy is better when administered as a continuous infusion

Adverse effects

Frequent: local reactions, pain, swelling, headache, urticaria.

Rare: gastrointestinal disturbance, hepatic and renal impairment, arrythmia, rashes, anaphylactic reactions. Opthalmological toxicity - reduced visual acuity, impaired color vision and night vision.

There is also an increased risk of infections due to Yersinia enterocolitica and Klebsiella pneumonia. In case of local reactions to the injection, dilution can be increased to more than 5ml of water for injection, with change in sites for injections and application of local steroid cream.

DFO is the chelator of choice for patients presenting with cardiac failure due to iron overload. It can also be used to reduce iron overload before conception or hematopoietic stem cell transplantation. Vitamin C increases the availability of iron, thereby increasing iron available for DFO to chelate. Vitamin C is administered concomitantly with DFO at a dose less than 2-3mg/kg/day.

When treating iron poisoning, DFO is administered as continuous IV infusion in neonates and children, initially up to 15 mg/kg/hour, max. 80 mg/kg in 24 hours, dose to be reduced after 4-6 hours.

Deferiprone

Deferiprone (DFP) is a bidentate oral iron chelator licensed for use in children above the age of 6 years. It is administered at a dose of 25 mg/kg 3 times a day; maximum 100 mg/kg per day. The main adverse effects include nausea, vomiting, abdominal pain and increased appetite. These effects are more frequent at the beginning of therapy and in most patients resolve within a few weeks of continued treatment. Also reported are reddish/brown urine, agranulocytosis, neutropenia and arthropathy predominantly affecting the knee, wrist and ankle joints. It is recommended to decrease the dose soon after the initial manifestation of joint symptoms and stop the drug in case of persistent symptoms. Neutropenia can occur in the first year of therapy necessitating complete blood count check every 2 to 3 weeks. DFP has greater efficacy in decreasing cardiac iron as compared to other chelators.

Deferasirox

Deferasirox (DFX) is a oral iron chelator licensed for use in children above two years of age. The drug is administered once a day. The dosage for a child 2-17 years: Initially 7-21 mg/kg once daily, dose adjusted according to serum-ferritin concentration and amount of transfused blood and adjusted in steps of 3.5-7 mg/kg every 3-6 months, maintenance dose adjusted according to serum-ferritin concentration; maximum 28 mg/kg per day.[13] The drug is preferably given before a meal.The drug has to be dispersed in water or juice. A glass or non metal container should be used for dissolving the medicine. The adverse effects include abdominal pain, pyrexia, headache, cough, diarrhea, vomiting, rash, nausea and increase in serum creatinine, rarely sensorineural deafness or hypoacusis. Adverse events requiring discontinuation of medication include abnormal LFT, drug induced hepatitis, skin rash, glycosuria and proteinuria, Henoch-Schonlein purpura, hyperactivity and insomnia, drug fever and cataract. Serum creatinine and SGPT need to be monitored regularly, preferably once in 2 to 3 months. In case of deranged values, the drug needs to be withheld and restarted when normalized and tolerated. Loss of zinc and calcium in the urine necessitates concomitant zinc and calcium supplements. DFX reduces liver iron concentration across all LIC values and is non-superior to DFO for chelating cardiac iron.

DFX film-coated tablets are a recent addition that are long-acting with decreased gastrointestinal adverse effects. The film-coated tablet can be administered after meals and does not require any specific preparation. The recommended pediatric dose is 14 mg/kg. It is, however, more expensive than the regular DFX.

Conclusion

Management of iron overload requires a thorough understanding of the iron chelator drugs, their dose adjustments in response to periodic monitoring of serum ferritin and later liver and cardiac iron estimations, transition to combination therapy and managing adverse effects. Optimal iron chelation is the key to health of children with conditions that cause iron overload.

Points to Remember

- ***Uncontrolled transfusional iron overload increases the risks of heart failure, endocrine damage, liver cirrhosis and hepatocellular carcinoma.***
- ***Chelation therapy is an effective treatment modality (but not ideal as yet) in improving survival, decreasing the risk of heart failure and decreasing morbidities from transfusional iron overload and should be started at least within 2 years of starting regular blood transfusions.***
- ***Response to chelation is dependent on the dose and the duration of exposure***
- ***Changes in body iron in response to transfusion and chelation can usually but not always be estimated from the trend in serum ferritin - Liver iron concentration (LIC) is better indicator of total body iron, and serum ferritin is an approximate marker of LIC.***
- ***Iron mediated tissue damage is often irreversible, and removal of iron deposited in tissues by chelation is slow - particularly after it has escaped the liver. Chelation of liver iron is faster than from the myocardium.***
- ***Heart iron accumulates later than liver iron, and is rare before the age of 8 years.***
- ***Over chelation increases side effects from chelation therapy, and doses should therefore be decreased as serum ferritin or liver iron levels fall (demonstrated most clearly with DFO)***
- ***The chelation regime must be tailored for the individual child and will vary with the clinical situation.***
- ***Chelation therapy will not be effective if it is not taken regularly - a key aspect of chelation management is to work with patients to ensure adherence.***

References

1. Porter JB. Practical management of iron overload. British J Haematol 2001; 115:239-252.
2. Esposito BP, Breuer W, Sirankapracha P, Pootrakul P, Hershko C, Cabantchik Z. Labile plasma iron in iron overload: redox activity and susceptibility to chelation. Blood 2003; 102(7):2670-2677.
3. Taher A, Cappellini MD, Vichinsky E, Galanello R, Piga A, Lawniczek T, et al. Efficacy and safety of deferasirox doses of >30 mg/kg per d in patients with transfusion-dependent anaemia and iron overload. British J Haematol 2009; 147:752-759.
4. Delea TE, Edelsberg J, Sofrygin O, Thomas SK, Baladi JF, Phatak PD. Coates TD. Consequences and costs of noncompliance with iron chelation therapy in patients with transfusion-dependent thalassemia: a literature review. Transfusion 2007; 47:1919-1929.
5. Kwiatkowski JL, Kim HY, Thompson AA, Quinn CT, Mueller BU, Odame I, et al. Chelation use and iron burden

in North American and British thalassemia patients: a report from the Thalassemia Longitudinal Cohort. Blood 2012; 119:2746-2753.

6. Bollig C, Schell LK, Rücker G, Allert R, Motschall E, Niemeyer CM, et al. Deferasirox for managing iron overload in people with thalassaemia. Cochrane Database of Systematic Reviews 2017, Issue 8. Art. No.: CD007476. DOI: 10.1002/14651858. CD007476.pub3.
7. Fisher SA, Brunskill SJ, Doree C, Chowdhury O, Gooding S, Roberts DJ. Oral deferiprone for iron chelation in people with thalassaemia. Cochrane Database of Systematic Reviews 2013, 8. Art. No.: CD004839. DOI: 10.1002/14651858. CD004839. pub3.
8. Neufeld EJ, Galanello R, Viprakasit V, Aydinok Y, Piga A, Harmatz P, et al. A phase 2 study of the safety, tolerability, and pharmacodynamics of FBS0701, a novel oral iron chelator, in transfusional iron overload. Blood 2012; 119: 3263-3268.
9. Hamilton JL, Kizhakkedathu JN. Polymeric nanocarriers for the treatment of systemic iron overload. Mol Cell Ther 2015; 3:3. doi:10.1186/s40591-015-0039-1.
10. Hoffbrand AV, Taher A, Capellini MD. How I treat transfusional iron overload. Blood 2012; 120: 3657-3669. doi: 10.1182/blood-2012-05-370098.
11. Chuang T, Li J, Weng T, Wu K, Chao Y. Combined chelation with high-dose deferiprone and deferoxamine to improve survival and restore cardiac function effectively in patients with transfusion-dependent thalassemia presenting severe cardiac complications. Ann Hematol 2020; 99(10):2289-2294.
12. Eghbali A, Shokri P, Afzal BR, Bagheri B. A 1-year randomized trial of deferasirox alone versus deferasirox and deferoxamine combination for the treatment of iron overload in thalassemia major. Transfus Apher Sci 2019; 58(4):429-433.
13. Deferasirox. In: British National Formulary for Children, BMJ group and Pharmaceutical Press, Tavistock Square, London, 2019-20; pp596-597.

CLIPPINGS

Association between proton pump inhibitor use and risk of asthma in children

The use of proton pump inhibitors (PPIs) in children has increased substantially in recent years, concurrently with emerging concerns that these drugs may increase the risk of asthma. Whether PPI use in the broad pediatric population is associated with increased risk of asthma is not known. This nationwide cohort study collected registry data in Sweden from January 1, 2007, to December 31, 2016. Children and adolescents 17 years or younger were matched by age and propensity score into 80 870 pairs of those who initiated PPI use and those who did not. Data were analyzed from February 1 to September 1, 2020. The primary analysis examined the risk of incident asthma with a median follow-up to 3 years.

Among the 80 870 pairs (63.0% girls), those who initiated PPI use had a higher incidence rate of asthma (21.8 events per 1000 person-years) compared with noninitiators (14.0 events per 1000 person-years), with a hazard ratio (HR) of 1.57 (95% CI, 1.49-1.64). The risk of asthma was significantly increased across all age groups and was highest for infants and toddlers with an HR of 1.83 (95% CI, 1.65-2.03) in the group younger than 6 months and 1.91 (95% CI, 1.65-2.22) in the group 6 months to younger than 2 years ($P < .001$ for interaction). The HRs for individual PPIs were 1.64 (95% CI, 1.50-1.79) for esomeprazole, 1.49 (95% CI, 1.25-1.78) for lansoprazole, 1.43 (95% CI, 1.35-1.51) for omeprazole, and 2.33 (95% CI, 1.30-4.18) for pantoprazole.

In this cohort study, initiation of PPI use compared with nonuse was associated with an increased risk of asthma in children.

Wang Y, Wintzell V, Ludvigsson JF, Svanström H, Pasternak B. Association Between Proton Pump Inhibitor Use and Risk of Asthma in Children. JAMA Pediatr. 2021; 175(4):394-403. doi:10.1001/jamapediatrics.2020.5710.

ADOLESCENCE

RELATIONSHIP COUNSELLING

***MKC Nair**
****Shyamal Kumar**
****Riya Lukose**

Abstract: *'Human relationship' has various phases. Counselling being a collaborative effort between the counselor and client, aims at identifying goals and potential solution to problems which causes emotional conflicts. A boy-girl relationship follows certain laws- 'laws of attraction, difference and self-image'. In this context, relationship counseling not only aims at identifying the problems but also provides insight into the type of relationship. Based on this knowledge, one can seek to improve communication and coping skills, strengthen self-esteem and promote behavioural changes and strong inter-personal relationships.*

Keywords: *Relationship, Counselling, Boy-Girl relationship, Love relationship*

The notion of "human relations" is one of the important theoretical movements of the 1960's and RE Miles was responsible for much of the work on crystalizing the notion of "human relations".[1] He shifted the focus from 'scientific management', which viewed people as part of a working machine, to the 'human relations approach' that changed the viewpoint from the task to the worker. For communication scholars, the human relations perspective sees communication as a tool that can be used by management to "buy" cooperation from subordinates. For psychologists, human relation includes an overview of basic psychological and cultural concepts related to human behaviour interactions and dealing with principles of communication, listening and conflict resolution, with an emphasis on skill development to improve relationships.

* Director,

** IAP Fellow in Developmental Pediatrics,
NIMS-Spectrum-Child Development Research Centre (CDRC),
NIMS Medicity Campus,
Thiruvananthapuram.

email: cdcmkc@gmail.com

Human relations counselling model

The human relations counselling model emphasizes on a client-centered helping relationship and the mutual identification of goals, objectives and intervention strategies that can ultimately be evaluated through the client's observable behavioural change. The approach uses a variety of techniques and strategies, but the major vehicle for change is the development and maintenance of a warm, empathic relationship with the client. In the context of a counselling relationship, Roger described the kind of nonthreatening environment necessary for individuals to integrate experiences into the self-structure through the six necessary and sufficient conditions for constructive personality change.[2]

1. Two persons should be in psychological contact.
2. The first, 'the client', is in a state of incongruence, being vulnerable or anxious.
3. The second person, 'the counselor', is congruent or integrated in the relationship.
4. The counselor experiences unconditional positive regard for the client.
5. The counselor experiences an empathic understanding of the client's internal frame of reference and endeavors to communicate this experience to the client.
6. The communication to the client of the counselor's empathic understanding and unconditional positive regard is to a minimal degree achieved.

- **Congruence** : Roger defined congruence as a close matching between the counselor's real experience, what is present in the counselor's awareness, and what is expressed to the client. Congruence includes the therapist's openness and attunement to her moment-by-moment experience and the counselor's genuine experience and expression of unconditional positive regard and empathy towards the client.[3]
- **Unconditional positive regard**: Roger defined unconditional positive regard as warm acceptance of every aspect of the client's experience. Unconditional positive regard reflects the counselor's fundamental belief in the self-actualizing tendency of the client.[3]

- **Empathic understanding**: Roger defined empathic understanding as a process of "entering the private perceptual world of the other and becoming thoroughly at home in it ...being sensitive, moment to moment, to the changing felt meanings which flow in this other person and ... communicating your sensing's of his/her world". [3]

The counsellor must learn when and how to use a battery of techniques and strategies with the same client in order to deal as fully as possible with the client's cognitive, affective and behavioural domains. The goals of counselling are to help the client become emotionally and cognitively aware of his/her responsibility and choices and translate this awareness into action. The helping relationship is the foundation of the helping process. As long as there is an effective helping relationship that communicates the helper's understanding, strength, and ability to give permission and protection to the client, flexibility to select, use and even fail with different strategies is possible. The communication skills required in this counselling model are the abilities to hear verbal messages, perceive nonverbal messages, and respond to these messages verbally and nonverbally.[4]

Assumptions of human relations counselling model

- Environment has a lot to do with everything a person goes through and some, especially with mental illness may not be capable of making their own sound decisions.
- Many of the problems people face are out of societal and systemic issues rather than inter or intrapersonal issues.
- Problems occur from unfinished issues from the past and it shapes who you are and the effects of which come years later, especially with reference to childhood issues.
- Young people are capable of learning new behaviours and eliminating the old ones, that they are able to reinforce themselves.
- Young people are capable of making their own decisions within their environmental factors.
- Young people are striving to meet their needs and those behaviours are purposeful and goal oriented.
- Young people want to feel good about themselves, they need confirmation from others.

Boy/girl relationship

A boy/girl relationship usually starts, when a boy and a girl meet and they develop an attraction or a feeling of liking towards each other and in many cases, the relationship in a school may not lead to courtship. The laws of boy-girl relationships include:

The law of attraction: We are attracted to that which is hard to get and we think little of that which is easily obtained. There is a strange fact about human nature. We value and prize that which is difficult to get and we treat lightly or despise that which is obtained with little or no effort on our part.

The law of difference: There is a basic difference in the way boys and girls think about love and are different in many ways, not only in the obvious, outward differences, but in their thinking as well. For example, the boy thinks of love in terms of sex; a girl thinks of love in terms of romance.

The law of self-image: We are controlled by the way we see ourselves inwardly. To put it another way, we are going to act out the way we see ourselves. If we see ourself as not being worth much, we are going to act that way. We all manage to remember all the "put downs" we have experienced.

Ways to have a healthy boy-girl relationship

In order to develop a good boy-girl relationship one has to love oneself, accept oneself as who he is, become good friend of oneself, be respectful to others, socialize and meet more friends, be cheerful always, greet acquaintances by first name and be socially pleasant.

- Being compassionate is understanding the feeling of the other family members, respecting the elders as well as the younger ones, thus developing their trust and confidence.
- Find balance between ones relationship and other aspects in life.
- Honesty builds trust and trust is an essential part of a happy relationship.
- In a healthy relationship, a person and his or her friends need to talk openly to one another.
- It is important to accept and be comfortable with what one has and who is engaged in a relationship.
- Respect means valuing the opinions, beliefs and ideas of one's friend.

The things that are essential for any relationships are; (i) trust - the most important ingredients of a happy and healthy relationship, (ii) respect - respecting the

individuality of partner, (iii) caring - love, attention and effective communication.

Types of relationships: The most common relationship types are (i) monogamous relationships, (ii) polyamorous relationships, (iii) open relationships, (iv) long-distance relationships, (v) casual sex relationships, (vi) 'friends with benefits' relationships, (vii) asexual relationships and (viii) true caring love relationships.

Different types of love relationships: Seven types of love include;

(i) Liking (intimacy)

(ii) Infatuation (passion)

(iii) Empty love (commitment without intimacy or passion)

(iv) Romantic love (passion + intimacy)

(v) Fatuous love (commitment + passion)

(vi) Companionate love (intimacy + commitment)

(vii) Consummate love (passion + intimacy + commitment)

Types of boy-girl relationships

1. Asexual relationship: Both partners are sexually attractive and get attracted to each other, but aren't interested in having sex (traditional Indian view).
2. Abusive relationship: One partner holds the reins and controls the other partner, either verbally or physically and may resort to hitting.
3. Co-dependent relationship: Too dependent on the partner, and completely rely on him/her to help you with decision making.
4. Complicated relationship: Both partners may know that things aren't perfect in love, because of a third person, or incompatibility, but have no idea how to fix it.
5. Controlling relationship: One partner plays a dominant role in the romance, while the other just follows the rules and starts getting frustrated and feeling helpless.
6. Friends with benefits: No strings attached; agreement between two people, where there's sexual intimacy and nothing more, both feeling insecure in the relationship.
7. Distracted relationship: Both partners are in love, but completely invisible to each other, being too focused on their careers, music, sports, etc.
8. Emotional relationship: A kind of secret affair with someone, being addicted to him/her and even willingly jeopardize own's perfect relationship.
9. May-December relationships: A relationship in which someone who's at least 10-15 years older or younger and hence needs to learn to deal with different expectations.
10. Held-by-loss relationship: Lost a lover or experienced a painful breakup and a rebound relationship developed with someone, just to fill the emptiness inside.
11. Imperfect relationship: Partner does not complain, because he/she has accepted the other person and life to be less than perfect, though not happy about it.
12. Insecure relationship: Both may lead their own independent lives and have own friends and one of the partner assumes that the other is cheating or is interested in someone else.
13. Long distance relationship: Both love each other and are connected to each other emotionally, but have only minimal physical intimacy.
14. Love-hate relationship: Crazy about each other, and yet, can't stand each other at times.
15. Negotiation relationship: Happy with each other with a lot of negotiations and compromises from both sides, just to keep the other partner happy.
16. Open relationship: Both partners are emotionally committed to each other, but not so sexually.
17. Pastime fling: Both are in love, but somewhere inside, are convinced that the relationship won't work out or last forever.
18. Sacrificial relationship: Unconditional love in its worst form, where one is truly in love, but the partner doesn't love with the same intensity, leading on to bitter fights.
19. Sexual affair: In a relationship only for sex with no emotional connection and just don't care about building the love and caring.
20. Toxic relationships: A relationship characterised by behaviours that are emotionally and physically damaging to the partner which affect self - esteem and drain energy.
21. Trophy relationship: Dating partner only because it makes one look better or gives something materialistic in return.
22. Truly compatible romance: Both are compatible, understand and accept each other for what both of them are; there is love in the air, and everyone else is envious of their relationship.

23. Unhappy relationship: The partners are not happy in the relationship, but are still staying back, not for love, but for something else.
24. Spiritual love relationship. There is little or no romance but lot of caring - heart doesn't rule, instead brain rules; no ecstasy, but lot of stability in marriage.

Top 10 - Teenage relationship problems include; (i) serious love or just a date, (ii) not aware how to tell your parents about your feelings (iii), limited money, (iv) jealousy and trust issues, (v) lack of maturity, (vi) parents, (vii) peer pressure, (viii) fear of losing first love, (ix) social media and (x) break up.

Eight ways a physical relationship before marriage affects relationship

1. Could be giving away all you have
2. Could get pregnant
3. Feel trapped
4. Focus on other responsibilities after marriage
5. Give up control
6. Might not go any further in a relationship
7. Relationship could end up being just about sex
8. Sex makes the relationship stronger

Although, it is true that the vast majority of those who had physical relationship before marriage are capable of safeguarding themselves emotionally, at least some of them may be troubled by distrust, guilt, low self-esteem, paranoia, scepticism, self-doubt, sexual dysfunction, shame and most importantly unsatisfactory sex.

Intervention strategies

The feasible intervention strategies include family life education at high school level, adolescent counselling, premarital and newly wed counselling, partner-relationship counselling (couple counselling) using partner-relationship assessment scale Trivandrum (PAST- abridged - 13 item and full version - 26 items).[5-8]

The goal of couple counselling is to build an existential base to their lives; identify and communicate their sense of purpose, priorities and values; what they hold to be sacred; missions, ethics, morality; philosophy of life and religion; legacy from their families and culture and meaning of how to move through time together. A sense of reality can be imparted in adolescent relationship counselling by the following aphorism "There will be lot of boys/girls to "love" you, but very few to take lifelong responsibility; multi-colour fantasy of youth will become black and white reality later on" – MKC Nair

Points to Remember

- ***The human relationship counselling model follows Roger's client-centered approach, where the client forms the core part of therapy process.***
- ***A boy-girl relationship follows certain laws- 'laws of attraction, difference and self-image'.***
- ***The boy-girl relationship can be of multiple types, and these have their own set of relationship issues.***
- ***A healthy boy-girl relationship is formed on the grounds of honesty, compassion, finding right balance, talking openly and having mutual respect.***
- ***Physical relationship before marriage can affect post marriage relationship.***

References

1. Miles RE. Human relations or human resources? Harv Bus Rev 1965; 43(4):148-157.
2. Rogers C. The necessary and sufficient conditions of therapeutic personality change. J Consult Psychol 1957; 21(2):95-103.
3. Bratton S, Purswell K, Jayne K. Play therapy - A child centered approach. In: Thompson HP, Fedewa AL. (Editors). Counseling and Psychotherapy with Children and Adolescents. 5th Edn. Hoboken, New Jersey:John Wiley & Sons, Inc; 2015; pp95-97.
4. Okun BF. Effective Helping-The Human Relations Counseling Model' (From Correctional Counseling and Treatment. Peter C Kratcoski See NCJ-74557). United States: Duxbury Press; 1981; pp213-222.
5. Nair MKC. Presenting Family life education. In: MKC Nair, Ranjan K. Pejaver (Eds). Adolescence and Family Life Education. 1st Edn. Bangalore: Prism Books; 2002; pp97-146.
6. Paul KM, Suresh KG. Guidance and Counseling in School setting. In: Nair MKC, Babu George, Indira MS, Leena Sumaraj (Eds). Adolescent Counselling. 1st Edn. New Delhi:Jaypee Brothers; 2016; pp73-87.
7. Nair MKC, Babu George, Leena Sumaraj. Premarital Guidance and support. In: MKC Nair, Paul Russell, Babu George, Leena Sumaraj (Eds). Adolescent Pediatrics. 1st Edn. Delhi: Noble Vision: 2017; pp202-213.
8. Mini G, Asha Kumari, Nair MKC. Development of a tool on partner relationship and estimation of prevalence of marital disharmony among married people. Ph.D Thesis Submitted to University of Kerala, 2020.

RADIOLOGY

CHEST RADIOGRAPHY IN PULMONARY TUBERCULOSIS

Raveendran J

Tuberculosis is a leading infectious disease in developing countries like India. Tuberculosis in children presents in a varied manner ranging from asymptomatic infection to life threatening disease. Diagnosing tuberculosis in children remains challenging. Radiological modalities play an important tool for diagnosing the condition. In children, the radiological findings are subtle and do not follow typical pattern as described in adults.

Chest x-ray is recommended as a first line investigation in all children with suspicion of tuberculosis. We will discuss in detail about the common radiological findings in pulmonary tuberculosis.

X-ray chest

Findings on imaging reflect the natural progression of infection from latent TB to primary pulmonary TB or disseminated disease depending on the response of the host and the clinical stage at evaluation. The National Tuberculosis Elimination Program (2019) classifies chest x-ray in TB suspects as

- Highly suggestive chest X-ray findings include a) Miliary shadows, b) Lymphadenopathy (hilar or mediastinal) or c) Chronic fibro-cavitary shadows.
- Non-specific chest X-ray findings refer to patterns other than highly suggestive radiological findings, like consolidations, non-homogenous shadows or bronchopneumonia.

Highly suggestive chest X-ray findings

a. Miliary tuberculosis

Miliary TB presents as small, diffuse, nodular opacities distributed evenly throughout the lung fields, characteristic of hematogenous dissemination which usually occurs in young children and immune compromised patients. Miliary nodules in children may vary from 1-2 mm to large coalescing patchy opacities. It is seen both in primary and post-primary tuberculosis (Fig.1).

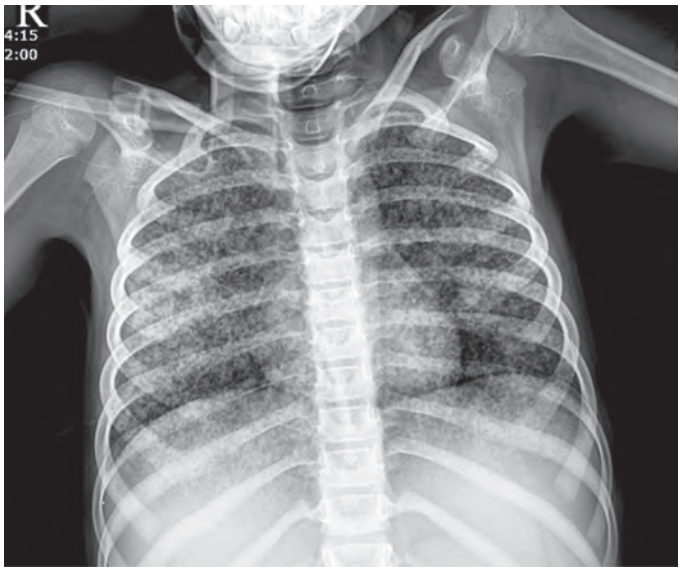


Fig.1.Chest radiograph: Multiple tiny nodular infiltrates distributed diffusely in bilateral lung fields, suggestive of miliary tuberculosis

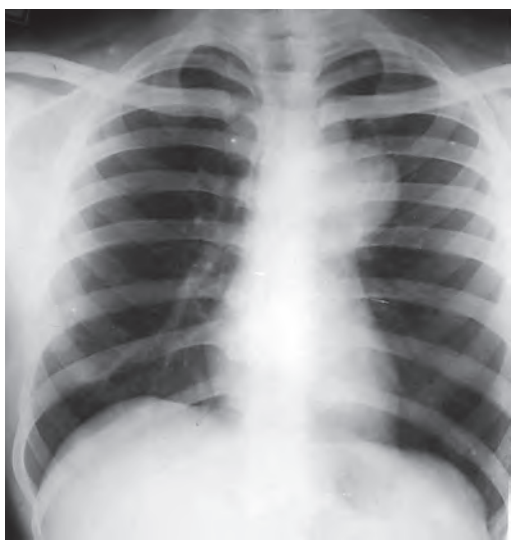


Fig.2. Chest radiograph: Hilar adenopathy

* Assistant Professor in Radiodiagnosis,
Department of Pediatric Radiology,
Institute of Child Health and Hospital for Children,
Chennai.

email: raveendran.jayabalan@gmail.com

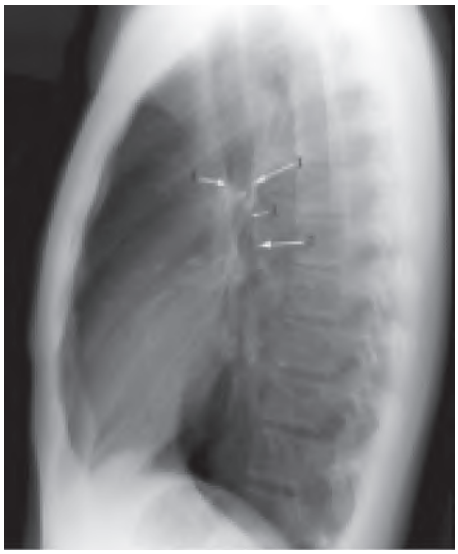


Fig.3a. inverted horseshoe configuration lateral chest X-ray.

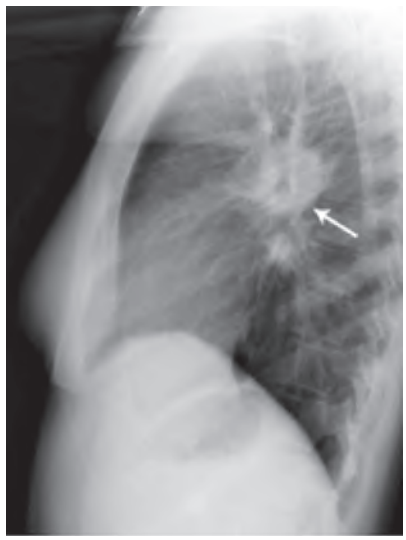


Fig.3b. Doughnut sign

(Source: Marshall GB, Farnquist B, MacGregor JH, Burrowes PW. Signs in Thoracic Imaging. J Thorac Imaging 2006; 21(1):76-90).

a. Lymphadenopathy (hilar or mediastinal)

Chest radiographs can demonstrate lymphadenopathy of the hilar and para-tracheal regions on the anteroposterior view and subcarinal lymphadenopathy on the lateral view (Fig.2). It usually appears as a lobulated density occupying the hilum and obliterating the hilar point (which should normally be a crisp V-shape meeting of large vessels), resulting in an outwardly convex appearance. The normal thymus and heart are relatively large in young children and therefore the mediastinal width and para-tracheal soft-tissue thickness are not parameters that should be evaluated in the detection of mediastinal lymphadenopathy in the younger age groups. An enlarged thymus is an important differential diagnosis when interpreting right paratracheal adenopathy. It is important to note, however, that the normal thymus does not compress or displace any structures.

Doughnut sign: The lateral X-ray can be a useful modality to detect subcarinal lymphadenopathy as it is usually obscured by the cardiac and mediastinal shadows in anteroposterior views.

On a normal lateral chest radiograph, the aortic arch and right and left pulmonary arteries are visualized in an "inverted horseshoe" configuration. In the presence of subcarinal lymphadenopathy, the inferior portion of the "horseshoe" fills in. The lymphadenopathy appears as a mass posterior to the bronchus intermedius and inferior to the tracheal bifurcation, completing the rounded hilar "doughnut" density (Fig.3a and Fig.3b).

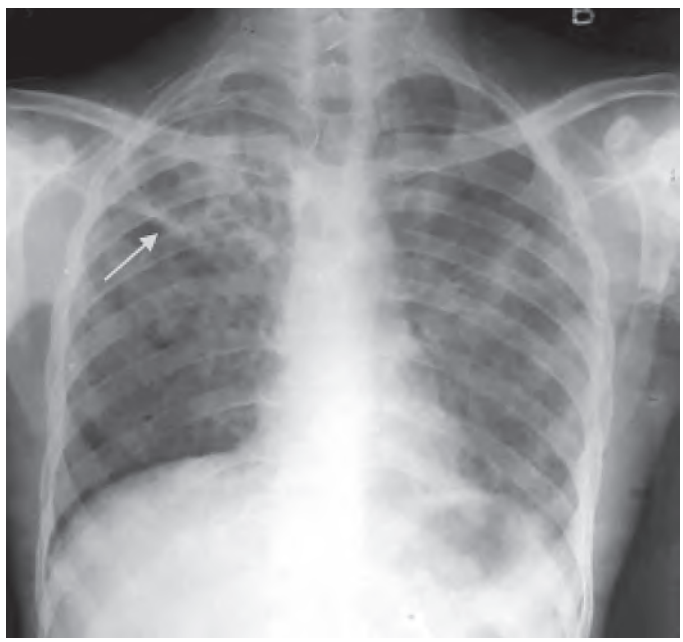


Fig.4. Chest radiograph: Chronic fibrocavitary tuberculosis in right upper lobe

a. Chronic fibrocavitary shadows

Cavitary tuberculosis is a radiological hallmark of post primary tuberculosis and hence is usually seen in older children and adolescents. Cavities are usually unilateral and seen in the upper lobes (Fig.4 & 5). The presence of cavities correlates with organism load, treatment outcome, risk of acquiring drug resistance, and infection risk posed to the community. In younger children, the cavities develop as a result of enlargement of the Ghon focus with eventual

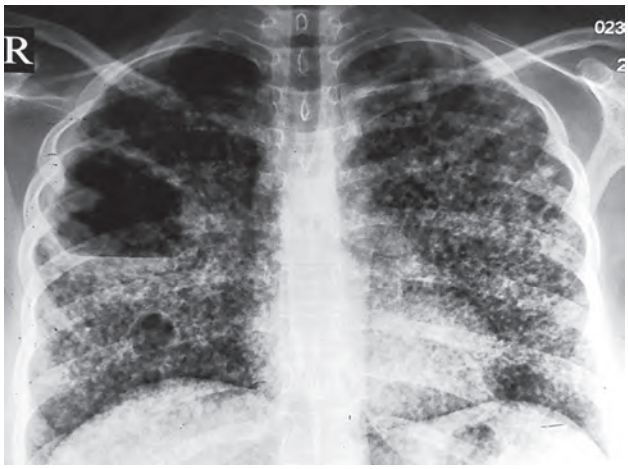


Fig.5. Chest radiograph: Chronic fibrocavitary tuberculosis with cavity showing air - fluid level and miliary nodules in both lung fields

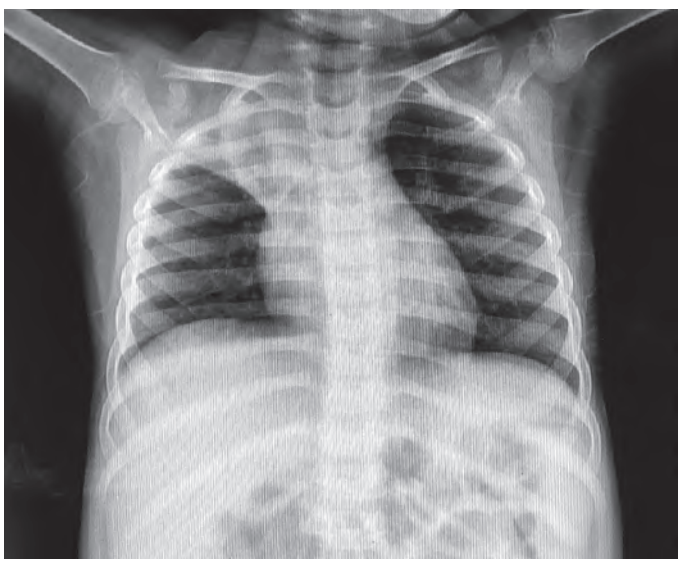


Fig.6. Chest radiograph: Right upper zone consolidation

caseous liquefaction at the centre of the focus. When this focus ruptures into an airway, cavitation occurs and endobronchial spread of disease results leading on to distal lobar involvement.

A cavity is usually thick walled (> 4mm), may rarely have air fluid level and can occur within an area of consolidation. It should be differentiated from a pneumatocele which is often post infectious, may be solitary or multiple. They are generally transient and the wall, if visible, is thin and regular.

Children with chest X-ray findings suggestive of tuberculosis can be subjected to cartridge based nucleic acid amplification test upfront (CBNAAT) GeneXpert.

Nonspecific radiological findings

Children with X-ray findings which are not suggestive of tuberculosis should be subjected to a course of antibiotics, initially. They include the following findings.

Consolidation: Air-space opacification with air bronchogram or central cavities is a frequent finding in chest radiographs (Fig.6).

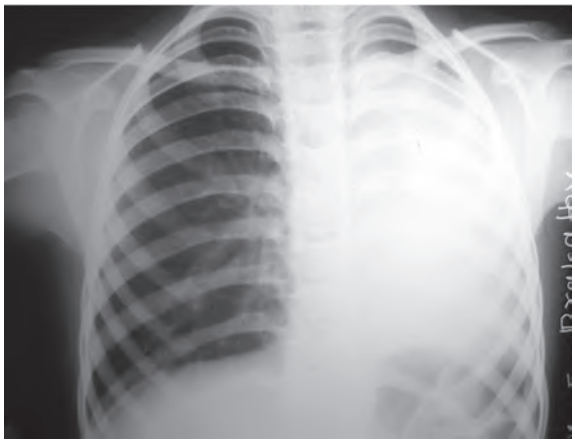


Fig.7. Chest radiograph: Collapse of entire left lung

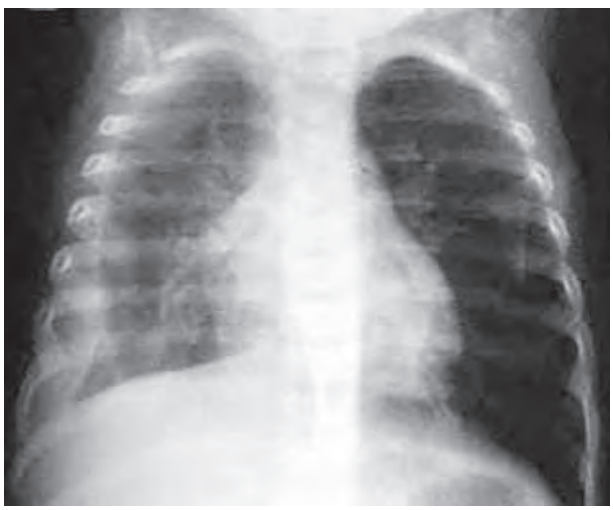


Fig.8. Chest radiograph: Right minimal pleural effusion

Collapse consolidation: This is caused by an enlarged hilar adenopathy causing complete compression of the adjacent bronchus causing collapse of the underlying lung segment. When the node undergoes caseous liquefaction and empties its contents into the bronchus, underlying consolidation is also noted. Chest X-ray shows collapse of the underlying lung (Fig.7). Partial compression of the bronchus by the node can cause unilateral hyperinflation due to the ball valve effect.

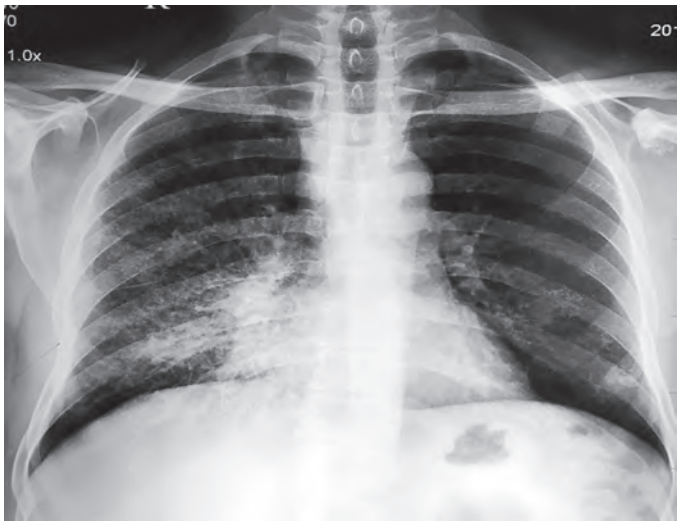


Fig.9. Chest radiograph: Bronchopneumonia

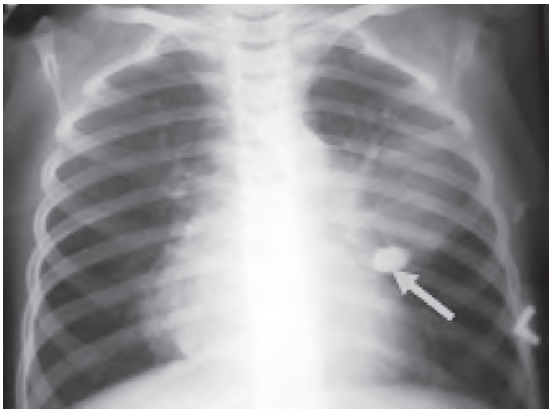


Fig.10. Chest radiograph: Calcified lymph node (arrow) with pneumonia left upper lobe

Pleural effusion: Tuberculous pleural effusion is frequently unilateral and can be of any size. It is associated with underlying parenchymal disease in 20% of cases (Fig.8).

Bronchopneumonia: Diffuse bilateral bronchopneumonia is a manifestation of progressive primary pulmonary tuberculosis. Infants presenting with acute pneumonia should be evaluated for tuberculosis when there is poor response to antibiotics and history of exposure to adult tuberculosis (Fig.9).

Ghon complex: The Ghon lesion is the initial tuberculous granuloma formed during primary infection and is not radiologically visible unless it calcifies - this occurs in up to 15% of cases. A Ghon focus with ipsilateral mediastinal lymphadenopathy is known as a Ghon complex. A calcified Ghon complex (Ghon lesion and ipsilateral mediastinal lymph node) is called a Ranke complex, which is radiologically detectable (Fig. 10).

We will discuss the CT and MRI imaging findings of tuberculosis in children in the subsequent issue.

CLIPPINGS

High-dose electronic media use in five-year-olds.

This 6 year study from Finland investigated the frequency of electronic media (e-media) usage by preschool children and the risks of high-dose e-media use on young children's psychosocial well-being. Psychosocial symptoms were determined using the parent-reported questionnaires Five-to-Fifteen (FTF) and the Strengths and Difficulties Questionnaire (SDQ).

The investigators found that 95% of the preschool children exceeded the daily recommended use of e-media set by health professionals. Their findings indicated that increased screen time at 5years of age is associated with a risk of multiple psychosocial symptoms (OR 1.53–2.18, 95%CI 1.05 to 3.34, $p<0.05$), while increased levels of e-media use at 18 months was only associated with FTF peer problems (OR 1.59, 95% CI 1.04 to 2.41, $p=0.03$). Moreover, high-dose use of electronic games at the age of 5years seem to be associated with fewer risks for psychosocial well-being than programme viewing, as it was only associated with SDQ hyperactivity (OR 1.65, 95% CI 1.08 to 2.51, $p=0.02$).

Niiranen J, Kiviruusu O,1 Vornanen R, Saarenpää-Heikkilä O, JuuliaPaavonen EJ. High-dose electronic media use in five-year-olds. BMJ Open 2021; 11:e040848. doi:10.1136/ bmjopen-2020-040848.

CASE REPORT

HYPERTRANSAMINASEMIA MASQUERADING AS WILSON DISEASE

***Riyaz A**

Abstract: *It is indeed very unfortunate that clinicians occasionally embark on the pursuit of expensive and invasive investigations, including liver biopsy, in the evaluation of children with isolated elevation of transaminases. Many of these children may be subsequently found to have various myopathies, including Duchenne muscular dystrophy. Superfluous testing can be avoided by following the basic principles of medicine like good history taking and meticulous clinical examination followed by relevant investigations.*

Keywords: *Hypertransaminasemia, Wilson disease, Duchenne muscular dystrophy, Gamma-glutamyl transpeptidase, Creatine kinase.*

A 5-year-old boy was evaluated by a pediatrician for a short febrile illness. As part of work up, LFT was done and the ALT was found to be 350 IU L and AST 480 IU /L. He recovered from his illness in a few days, but his transaminases repeated twice after 2 weeks, at different laboratories showed persistently raised values. Hence, he was referred to a higher center by his pediatrician to rule out any underlying chronic liver disease. There he was evaluated in detail and he was found to have grade 3 PEM according to IAP classification and mild pallor, but no icterus or edema. His liver was palpable 3 cm below the right costal margin, soft in consistency; no splenomegaly. His Hb was 9.8g/dL, total leukocyte count 6500/microliter, platelet 2,80,000/microliter and albumin 2.6 g /dL. AST was 420 IU/L (normal: 0-35), ALT 380 IU/L (normal: 0-40) and serum bilirubin 0.5mg/dL. Viral markers and autoimmune hepatitis markers were negative. His serum ferritin level was very low and peripheral smear was suggestive of iron deficiency anemia. His serum ceruloplasmin was 18 mg/dL (normal 20-40 mg/dL). Slit lamp examination was negative for KF ring. USG showed grade 1 fatty liver. Urine 24-hour copper done without penicillamine was inconclusive and liver biopsy was suggestive of fatty liver. Based on these, he was diagnosed to have Wilson disease (WD) and started on D-penicillamine along with the usual supportive measures.

He was referred to our center by the pediatrician who had seen him first as his transaminases continued to be high even after one year of treatment as suspected WD. He was the third child of non-consanguineous parents. There was no history suggestive of acute or chronic liver disease in the family. His motor milestones were delayed, having first walked at the age of 2 years. He was evaluated by a pediatric neurologist for this and was advised physiotherapy. His 7-year-old sister was normal while his 2-year-old brother had only just started walking with support.

He did not have jaundice or any signs of chronic liver disease. His calf muscles, deltoids, biceps and infraspinatus appeared bulky and Gowers sign and valley sign were positive (Fig.1). His gamma-glutamyl transpeptidase (GGT) was normal and creatinine kinase (CK) was 20,050 IU/L (normal: 20-200). CK of his sister was normal while that of his brother was also high (15,000 IU/L). These features helped us to make a diagnosis of DMD. He was referred to a Neurologist and geneticist who later confirmed the diagnosis of Duchenne muscular dystrophy (DMD).

Discussion

This child with hypertransaminasemia was diagnosed to have WD on the basis of hepatomegaly, low levels of

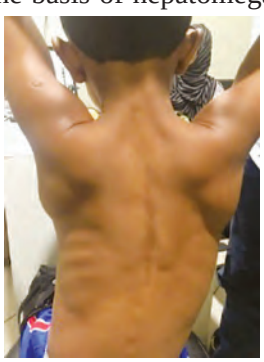


Fig.1. Hypertrophied deltoid and infraspinatus muscles in DMD

* Professor & Head of Pediatric Gastroenterology, KMCT Medical College, Calicut, Kerala.
email: riyazped@gmail.com

serum albumin and ceruloplasmin and fatty liver by USG and liver biopsy. But he also had grade 3 PEM which could explain all these features including low serum ceruloplasmin. His pallor was due to iron deficiency anemia, a part of his PEM, while hemolytic anemia, if present, would have been a point in favor of WD. His serum ferritin was very low and peripheral smear was suggestive of IDA. Further investigations for IDA could not be done due to financial constraints. The pre-penicillamine 24-hour urine copper analysis may be normal or inconclusive in WD, in which case a post-penicillamine 24-hour urine copper analysis may have been helpful. However, this test is unreliable in pre-symptomatic children with WD and may also be abnormal in heterozygotes of WD. The normal GGT along with the very high CK in the child as well as his asymptomatic younger brother, helped us to suspect DMD.

Transaminases include alanine aminotransferase (ALT) and aspartate aminotransferase (AST). They are normally present in circulation at low levels. They are intracellular enzymes synthesized mainly by the hepatocytes and increased levels in serum usually suggest hepatocellular damage. An elevated serum ALT level is more liver-specific compared to AST. However, there are a few conditions in which AST: ALT ratio (De Ritis ratio) is > 2:1 These include WD, alcoholic liver disease and various myopathies. AST is concentrated in the mitochondria of hepatocytes unlike ALT which is more in the cytosol. In WD and alcoholic liver disease (which includes alcoholic hepatitis occasionally seen in adolescents), the brunt of damage is borne by the mitochondria resulting in a disporportionate release of AST compared to ALT. The concentration of AST is more in skeletal muscles than ALT and hence in any condition that causes myocyte damage like primary muscle disease, myositis and strenuous exercise, AST level is more than that of ALT. In our patient, AST was persistently more than ALT and this may have been one reason why the pediatrician thought of the possibility of WD.[1,2]

The serum levels of aminotransferases depend not only on the tissue of origin, but also on their half-lives, which is longer for ALT (48 hours) than AST (18 hours). Thus, occasionally in muscle diseases such as DMD, serum AST and ALT may be elevated to the same degree.[1]

Macro-AST is a high molecular mass complex of this enzyme with immunoglobulins especially IgG, or other proteins. This leads to reduced plasma clearance and prolonged half-life of AST which is thus retained in the plasma. This is a benign condition which is not due to liver disease or muscle disease. Macro-AST can be confirmed by adding polyethylene glycol to the serum which results in precipitation of macro-AST complexes, while the unbound enzyme stays in the supernatant.[3]

GGT is a membrane-bound enzyme produced primarily in the liver, with little or none produced from skeletal muscle. It is one of the most sensitive tests for the presence of hepatobiliary disease as it is almost always elevated in established, or even in incipient liver disease. A normal GGT along with the indiscriminate elevation of other enzymes including AST and ALT points to a muscle disease rather than a liver disease.[4,5] Thus, GGT is a reliable biomarker which helps to distinguish liver disease from muscle disease and helps avoid a liver biopsy.[6]

CK is a specific and inexpensive indicator of muscle disease. Its level reaches 100 times higher in muscles than in hepatocytes after the onset or progression of DMD.[7] Hence, CK should be included in the diagnostic algorithm of children with isolated hypertransaminasemia, before embarking on invasive investigations like liver biopsy.[8]

A large number of children with isolated hypertransaminasemia ultimately turn out to have DMD. In a study, it was found that almost 20% of such children had undergone liver biopsy. This approach is detrimental as it not only leads to unnecessary potentially dangerous invasive procedures but also delays the correct diagnosis and treatment of the underlying disease.[9,10]

References

1. Riyaz A. Pediatric Gastroenterology and Hepatology. 4thedn. Hyderabad. Paras Medical publishers; 2019; pp 344-362.
2. Stenhammar L, Klintberg B, Tevebring J, Henriksson KG. Muscular dystrophy misdiagnosed as hepatic disease in a child with celiac disease. Acta Pediatr 1995; 84:707-708.
3. Caropreso M, Fortunato G, Lenta S, Palmieri D, Esposito M, Vitale DF, et al. Prevalence and long-term course of macro-aspartate aminotransferase in children. J Pediatr 2009; 154:744-748.
4. Rosales XQ, Chu ML, Shilling C, Wall RNC, Pastores GM, Mendell JR. Fidelity of Gamma-Glutamyl Transferase (GGT) in Differentiating Skeletal Muscle From Liver Damage. J Child Neurol 2008; 23(7):748-751.
5. Ulgen PA, Dirik E, Ercal D. Letters to the Editor. Indian Pediatrics 2002; 39:210-211.
6. Kohli R, Harris DC, Whitington PF. Relative elevations of serum alanine and aspartate aminotransferase in muscular dystrophy. J Pediatr Gastroenterol Nutr 2005; 41:121-124.

7. McMillan HJ, Gregas M, Darras BT, Kang PB. Serum transaminase levels in boys with Duchenne and Becker muscular dystrophy. Pediatrics 2011; 127:132-136.
8. Wright MA, Yang ML, Parsons JA, Westfall JM, Yee AS. Consider muscle disease in children with elevated transaminase. J Am Board Fam Med 2012; 25:536-540.
9. Veropalumbo C, Del Giudice E, Esposito G, Maddaluno S, Ruggiero L, Vajro P. Aminotransferases and muscular diseases: a disregarded lesson. Case reports and review of the literature. J Pediatr Child Health 2012; 48:886-890.
10. Ramachandran J, Sajith KG All that glitters is not gold: Elevated liver enzymes do not mean liver disease always. Indian J Gastroenterol. 2014; 33(5):476-477.

CLIPPINGS

Achieving accurate laboratory diagnosis of typhoid fever

Currently available serological diagnostic methods for typhoid fever on the clinical utility of Tubex TF as an alternative to the Widal or Typhidot test.

A qualitative analysis was done to determine various serological tests used for typhoid fever diagnosis with emphasis on TubexTF (inhibition magnetic binding immunoassay)in comparison to the Widal of Typhidot test. Further, a meta-analysis was performed to obtain a pooled estimate of diagnostic accuracy (sensitivity and specificity) using different analysis models. A total of sixteen studies was included in the qualitative analysis. Further screening of these studies yielded ten studies that were used for the meta-analysis.

The sensitivity/specificity range of different commonly used serological tests in typhoid patients is between 55-100%/58-100% for TubexTF, 54-67%/54-95% for Typhidot and 32-95%/4-98% for the Widal test. As for the pooled meta-analysis estimates, the Tubex TF showed superior results when differentiating individuals with febrile illness of unknown origin from those with typhoid fever.

Overall, the results of this review and meta-analysis suggest that the Tubex TF is more advantageous to use as a serological test for typhoid fever diagnosis due its accuracy and simplicity. However, further studies are still needed .

Bundalian R Jr. Valenzuela M, Tiongcoc RE.Achieving accurate laboratory diagnosis of typhoid fever: A review and meta-analysis of Tubex TF clinical performance. Pathog Glob Health. 2019; 113(7):297-308. Published online 2019 Nov 28. doi: 10.1080/20477724.2019.1695081.PMCID: PMC7006692.PMID: 31778097.

Initial chest radiographs and artificial intelligence (AI) predict clinical outcomes in COVID-19 patients: analysis of 697 Italian patients.

This retrospective single-center study of adult patients presenting to the emergency department during Feb, Apr 2020, with SARS-CoV-2 infection confirmed on real-time (RT-PCR). Initial CXRs obtained on ED presentation were evaluated by a deep learning artificial intelligence (AI) system and compared with the Radiographic Assessment of Lung Edema (RALE) score, calculated by two experienced radiologists. Death and critical COVID-19 (admission to intensive care unit (ICU) or deaths occurring before ICU admission) were identified as clinical outcomes. Results Six hundred ninety-seven 697 patients were included.: mean age n age 62 years (IQR 52-75). Multivariate analyses adjusting for demographics and comorbidities showed that an AI system-based score ≥ 30 on the initial CXR was an independent predictor both for mortality and critical COVID-19 (HR 3.40 (95% CI 2.35-4.94; $p < 0.001$)). Other independent predictors were RALE score, older age, male sex, coronary artery disease, COPD, and neurodegenerative disease.

Conclusion: Our study has shown that initial CXR's severity assessed by a deep learning AI system may have prognostic value in COVID-19 patients, with a performance comparable to a radiologist-assessed score.

Mushtaq J, Pennella R, Lavalle S, Colarieti A, Steidler S, MartinenghiCMA, et al. Initial chest radiographs and artificial intelligence (AI) predict clinical outcomes in COVID-19 patients: Analysis of 697 Italian patients. EurRadiol 2021; 31:1770-1779.

CASE REPORT

MYXEDEMA COMA IN A CHILD WITH DOWN'S SYNDROME

*Suchitra Sivadas
*Gayathri Sajeevan
*Sajitha S
*Jayakumar C
**Nisha Bhavani

Abstract: *Myxedema coma is a rare condition characterised by severe hypothyroidism leading to depressed mental status, hypothermia and multiorgan dysfunction as a result of reduced circulating levels of thyroid hormones. We present here a boy with Down syndrome who was admitted with progressive lethargy, hypotension, and hypothermia who was diagnosed to have myxedema coma and managed appropriately. Treatment includes ICU care, replacement with thyroxine, intravenous steroids and supportive measures. Worldwide there are only very few case reports of children with Down syndrome presenting as myxedema coma. The condition requires a high index of suspicion, prompt diagnosis and treatment, as it is potentially life threatening.*

Keywords: *Hypothyroidism, Downs syndrome, Child.*

Myxedema coma is a severe life threatening, but potentially reversible form of decompensated hypothyroidism in which patients exhibit multiple organ abnormalities and progressive mental retardation.[1] Infections and discontinuation of thyroid supplements are the major precipitating factors. Low intracellular T3 leads to cardiogenic shock, respiratory depression, hypothermia and coma.[2] Patients are identified on the basis of high index of suspicion with a careful history and examination focused on features of hypothyroidism and precipitating factors.[3] Here we describe a 11-year-old male child with Downs syndrome and autoimmune thyroiditis who presented with myxedema coma.

* Department of Pediatrics

** Department of Endocrinology,
Amrita Institute of Medical Sciences,
Kochi.

email : suchudas2482@gmail.com

Case details

The patient was a 11 year 9-month-old boy with Down syndrome who presented to the emergency department of the hospital for the first time with progressive lethargy and sleepiness of 2 weeks, petechial rashes over the body of 1 week and fever with cough of 2 weeks duration. He had seizure disorder, was on valproate, but not on regular follow up. As the seizure frequency increased, the dose of valproate was increased by father of the child on his own to 48mg/kg/day 6 months back.

At admission, the child was stuporous, hypothermic (axillary temperature 95° F), bradycardic, hypotensive, hypoventilating with a saturation of 85% in room air. He had phenotypical features of Down syndrome with severe pallor, multiple petechiae all over the body. His weight was 25 kg (5th centile), height was 120 cm (<3rd centile) and OFC was 48 cm (<3rd centile). His SMR was stage1(preadolescent). There was no thyroid swelling clinically. He had features of left sided pneumonia. His GCS was 11/15 and there were no signs of meningeal irritation. Capillary blood glucose was normal (86 mg %). His complete blood counts showed bicytopenia (Hb-8.2 g/dl, total counts-9300, with neutrophils 52%, lymphocytes 20%, platelet count 30000) with elevated inflammatory markers (C reactive protein 151.9mg/dl, procalcitonin 24.26 ng/ml). Peripheral smear showed features of macrocytic anemia with thrombocytopenia. Serum vitamin B12 was normal. Serum folate levels could not be done. He was started on BIPAP, broad spectrum IV antibiotics and hemodynamic support. Further investigations showed hyponatremia (Sodium-130meq/L), low free T4(0.18ng/dl), significantly elevated TSH (>100 uIU/ml), high anti TPO antibodies (96.8U/ml) and low serum cortisol(6.24ug/dL). ECG showed bradycardia with low voltage complexes. Echo was normal. Pediatric endocrine consultation was availed, myxedema coma was considered and he was started on oral thyroxine at 150 mcg/day through nasogastric tube, following a stress dose of IV hydrocortisone. As the serum valproate was found to be in the toxic range (110.7mcg/ml), valproate induced bicytopenia was considered and valproate was stopped. His sensorium and vitals improved rapidly and hematological parameters normalised. The dose of

thyroxine was rapidly brought down to 75 mcg/day. The parents were counselled regarding self-administration of drugs. On follow up after 1 month, his free T4 was 2.47ng/dL, TSH was 0.39uIU/ml. The dose of thyroxine was reduced to 62.5 mcg/day and he is kept on follow up. Ultrasound thyroid was planned for, at review.

Discussion

The incidence of hypothyroidism in Down syndrome at birth is 1% (28 times more than the general population) with 0.7% having persistent hypothyroidism and 0.3% having transient forms.[4] Myxedema coma results from decompensation of long-standing untreated hypothyroidism. There are few reports of myxedema coma in children with Down syndrome and this is a rare presentation of the same. Such patients should be admitted to an intensive care unit for vigorous pulmonary and cardiovascular support.[5] Most authorities recommend treatment with intravenous levothyroxine (T_4) as opposed to intravenous liothyronine (T_3).[6] Because of the possibility of secondary hypothyroidism and associated hypopituitarism, hydrocortisone should be administered at a dosage of 100 mcg every eight hours[7] until adrenal insufficiency has been ruled out. Failure to treat with hydrocortisone in the face of adrenal insufficiency may result in the precipitation of adrenal crisis. The three essential elements for the diagnosis of myxedema coma include altered mental status, defective thermoregulation and a precipitating event or illness; all of these were present in our patient. Also, very high TSH, low T3 and T4 and the rapid response to the treatment with levothyroxine confirmed the diagnosis.[8] Though valproate has been reported to cause mostly subclinical hypothyroidism, this degree of profound hypothyroidism and myxedema coma is rare. Serum levels of valproate were in the toxic range. However Folic acid levels could not be estimated. Hence valproate induced macrocytic anemia due to folate antagonism though a possibility, was not proven.

References

1. Jordan RM. Myxedema coma: Pathophysiology, therapy and factors affecting prognosis. Med Clin North Am 1995; 79:185-94.
2. Nicoloff JT, Lo Presti JS. Myxedema coma. A form of decompensated hypothyroidism. Endocrinol Metab Clin North Am 1993; 22:279-90.
3. Kwaku MP, Burman KD. Myxedema coma. J Intens Care Med 2007; 22:224-31.
4. Coleman M. Thyroid dysfunction in Down syndrome: A review. Down Syndrome Research and Practice 1994; 2(3):112-115.
5. Thompson MD, Henry RK. Myxedema coma secondary to central hypothyroidism: A rare but real cause of altered mental status in pediatrics. Horm Res Pediatr 2016; 87: 350-353.
6. Root JM, Vargas M, Garibaldi LR, Saladino RA. Pediatric patient with altered mental status and hypoxemia. Pediatric Emergency Care July 2017; 33(7):486-488.
7. Salomo LH, Laursen AH, Reiter N, Rasmussen UF. Myxedema coma: an almost forgotten, yet still existing cause of multiorgan failure. BMJ Case Rep 2014; 2014:bcr2013203223.
8. Bansal D, Nanda A, Gupta E, Croker M, Williams ML, Bacchus A, et al. Myxedema coma in a patient with Down's syndrome. J Ark Med Soc 2006 Nov;103(5):112-113.

CLIPPINGS

Artificial intelligence-assisted auscultation in detecting congenital heart disease.

This study evaluates the accuracy of artificial intelligence-assisted auscultation

In total, 1362 patients with CHD were enrolled in the study (mean age - 2.4 ± 3.1 years and 46% female). The samples of their heart sounds were recorded and uploaded to the platform using a digital stethoscope. By the platform, both remote auscultation by a team of experienced cardiologists from Shanghai Children's Medical Center and automatic auscultation of the heart sound samples were conducted and analysed. Compared to face-to-face auscultation, remote auscultation detected abnormal heart sound with 98% sensitivity, 91% specificity, 97% accuracy and kappa coefficient 0.87. AI-AA demonstrated 97% sensitivity, 89% specificity, 96% accuracy, and kappa coefficient 0.84.

Conclusions The remote auscultations and automatic auscultations, using the AI-AA platform, reported high auscultation accuracy in detecting abnormal heart sound and showed excellent concordance

Lv J, Dong B, Lei H, Shi G, Wang H, Zhu F, et al. Artificial intelligence-assisted auscultation in detecting congenital heart disease. Eur heart J - Digital health. doi:10.1093/ehjdh/ztaa017. Jan 2021

CASE VIGNETTE

SUBCUTANEOUS ZYGOMYCOSIS

***Ashitha K. Unny**
***Siddharth Pandian**
****Seyed Rabia**
*****Gopinathan Kathirvelu**
******Senthil G. Kamaraj**

A 2-year-old boy from Thiruvallur, Tamilnadu came with complaints of lesion over his upper back since 5 months of age which started as a small swelling of size approximately 2x2 cms. In the past, he underwent incision and drainage of the swelling elsewhere. Following this, he had developed multiple small swellings centered upon the previous scar site. The swellings merged to form a single large lesion over the interscapular region at the level of the upper thoracic spine. On examination he had a swelling roughly of size 10x8 cms with irregular borders and extending about 2 cms and 6 cms left and right lateral to the thoracic spine respectively along its maximum diameter. (Fig.1) The swelling was not warm, non-tender, firm, indurated and was attached to the overlying skin which was looking normal. Surrounding lymph nodes were not palpable. MRI of the lesions showed diffuse plaque which is a T2W hyperintense lesion with restricted diffusion and interspersed fat signals. No intramuscular or intraspinal extensions and no significant arterial feeders were observed. The findings were suggestive of a tumor of soft tissue origin, possibly an involuting hemangioma or a neurofibroma.

Hence, a wedge biopsy of the lesion was planned to confirm the diagnosis. An elliptical incision was made over the most prominent part of the swelling at the lower right border of the diffuse lesion. The specimen included the lesion (1cm size) along with the overlying skin for histopathological examination, AFB and Gram staining and Gene Xpert for TB bacilli. The lab reports for tuberculosis, were all proved negative. Basic investigations like CBC were normal. The lesion was confirmed to be of fungal origin on HPE which showed multiple granulomas composed of epithelioid histiocytes and giant cells in deep dermis surrounded by sheets of eosinophils.

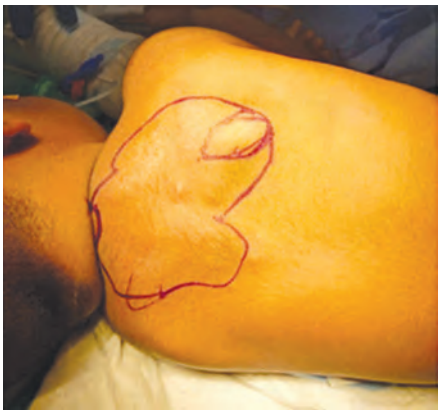


Fig.1. Clinical picture at presentation

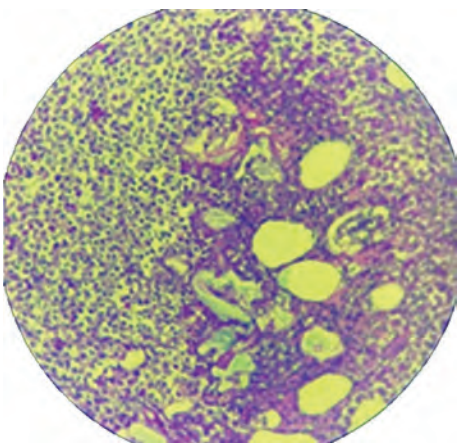


Fig.2. PAS stain showing actual fungi surrounded by Splendore hoeppli reaction (pink deposits). This phenomenon (asteroid bodies) is characterized by microorganisms (fungi, bacteria and parasites) or biologically inert substances surrounded by radiating intensely eosinophilic material

* Pediatric Surgery Resident
** Consultant Pathologist, Department of Lab Services, CHILDS Trust Medical Research Foundation, Kanchi Kamakoti CHILDS Trust Hospital, Chennai.
*** Prof of Radiodiagnosis, Department of Radiodiagnosis, Kilpauk Medical college, Chennai.
**** Senior consultantPediatric Surgeon, Department of Pediatric Surgery, CHILDS Trust Medical Research Foundation, Kanchi Kamakoti CHILDS Trust Hospital, Chennai.
email: ashitha.aku@gmail.com

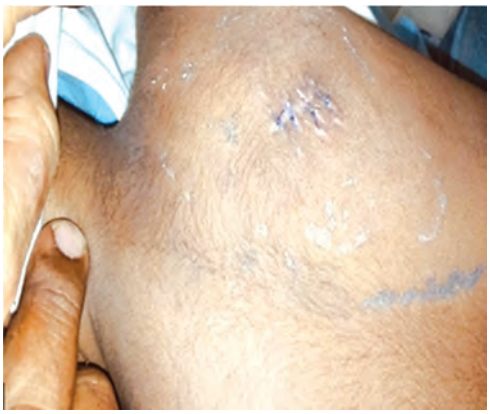


Fig.3. Clinical picture on follow up

Broad, infrequently septated fungal hyphal elements with surrounding Splendore-hoeppli phenomenon confirmed subcutaneous Zygomycosis (Fig.2). PAS and GMS stains also showed positive for fungal hyphae. Culture of the body fluids was not done. HIV serology was negative. The child was started on oral Itraconazole at 5 mg/kg/ day. After completion of 6 weeks of therapy, the lesion has shown signs of resolution to reach one-third of its original size (Fig.3).

Subcutaneous Zygomycosis is a rare fungal infection caused by Basidiobolus ranarum which belongs to Entomophthoraceae family.[1] It is a filamentous fungus present in soil, decaying fruits and vegetable matter as well as in the gut (dung) of various insectivorous reptiles (lizards and chameleon), amphibians (toads) and mammals (bats and kangaroos).[2] The portal of entry is believed to be through skin after insect bites, scratches and minor cuts either superficial structures like skin or might even spread amidst the deeper viscera like GIT. Though predominantly a disease of adults, there are reports of such lesions in infants. Boys from low socioeconomic background are commonly affected. In contrast, our patient was from a higher income group. They can be mistaken for soft tissue tumors, Burkitt lymphoma, cutaneous tuberculosis or other tropical infections.[3] These lesions may also mimic abscesses and often incised as in this boy. In this boy, embryonal type of rhabdomyosarcoma (ERMS) was considered an important differential diagnosis considering his age.

References

1. Thotan SP, Kumar V, Gupta A, Mallya A, Rao S. Subcutaneous phycomycosis-fungal infection mimicking a soft tissue tumor: a case report and review of literature. J Trop Pediatr 2010; 56(1):65-66. doi: 10.1093/tropej/fmp049.
2. Mathew R, Kumaravel S, Kuruvilla S, Varghese RG, Shashikala, Srinivasan S, et al. Successful treatment of extensive basidiobolomycosis with oral itraconazole in a child. Int J Dermatol 2005; 44(7):572-575. doi: 10.1111/j.1365-4632.2004.02419.x.
3. Gummadi GK, Pai BS, Nayak UKS, Prakash PY, Pai K. Basidiobolomycosis mistaken for cutaneous tuberculosis. Indian J Dermatol Venereol Leprol 2019; 85(2):236. doi: 10.4103/ijdvl.IJDVL_16_17. PMID: 29536980.

CLIPPINGS

SARS-CoV-2 infection of the oral cavity and saliva.

Despite signs of infection-including taste loss, dry mouth and mucosal lesions such as ulcerations, enanthema and macules-the involvement of the oral cavity in coronavirus disease 2019 (COVID-19) is poorly understood. To address this, the authors generated and analyzed two single-cell RNA sequencing datasets of the human minor salivary glands and gingiva (9 samples, 13,824 cells), identifying 50 cell clusters. Utilizing RNA sequencing and expression assessments, the authors of this study demonstrated that SARS-CoV-2 can infect and replicate within the glands and mucosa of the human oral cavity. Additionally, the authors found that saliva can serve as a vector for transmission of the virus in asymptomatic patients, and salivary viral load correlated with the degree of symptoms, namely loss of taste.

Prior to this study, it was unknown if SARS-CoV-2 could infect and replicate in the oral cavity. In addition to implicating the oral cavity as a potential source for transmission, this study indicates the need for additional evaluation of the interplay between the nasal and oral cavities in SARS-CoV-2 pathogenesis.

Huang N, Pérez P, Kato T, Mikami Y, Okuda K, Gilmore RC, et al. SARS-CoV-2 infection of the oral cavity and saliva. Nat Med 2021. https://doi.org/10.1038/s41591-021-01296-8.

CASE VIGNETTE

A RARE CAUSE OF OPEN ANTERIOR FONTANELLE IN A TODDLER

***Sridevi A. Naaraayan**
****Sharuka R**

A 2 year and 3 months old boy presented to the hospital for fever, cough and cold which was diagnosed as pneumonia and treated appropriately. He was incidentally noted to have a wide anterior fontanelle measuring 5x5 cm which was flushed with surface and pulsatile. Records revealed macrocephaly with a head circumference of 37cm at the time of birth. MRI brain done during neonatal period was reported to be normal. He had a slight delay in attaining motor milestones and started walking at around 18 months. His present head circumference was 49cm which was within normal limits. There was no occipital bulge or any other dysmorphic feature. Since his vitamin D status and thyroid profile were normal, MRI brain was done which showed cystic dilatation in posterior fossa with enlarged fourth ventricle communicating with it, normal morphology of vermis, with a tegmentovermian angle of 25º which are suggestive of Blake pouch cyst (Fig.1). As he did not have any features of raised intracranial pressure, he was advised regular follow up.

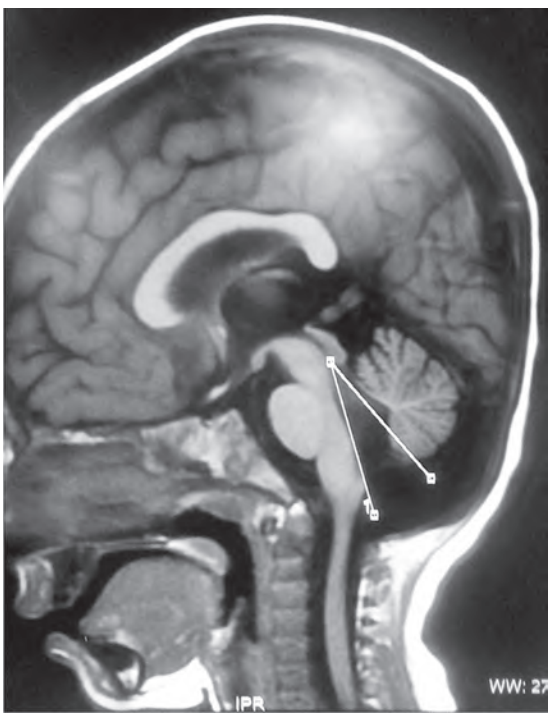


Fig.1. MRI brain showing blake pouch cyst with wide tegmento vermian angle

Blake pouch is a normal transient structure during embryological development. It regresses usually by 12 weeks of gestation when it starts fenestrating to form foramen of Magendie. If there is fenestration failure, it leads to Blake pouch cyst.[1] It is named after Joseph A. Blake (1864-1937), an American physician and persistent Blake's pouch cyst is considered as an independent entity within the Dandy Walker Complex (DWC). It is one of the causes of posterior fossa cyst and differentiation is radiological, rather than clinical. The main differentiating feature between Blake pouch cyst and Dandy Walker Malformation is the presence of normal vermis in former and hypoplastic vermis in the latter. Two causes of posterior fossa cyst with a normal cerebellum are mega cisterna magna and blake pouch cyst, the differentiating feature being wide tegmentovermian angle in the latter.[2] Although blake pouch cyst can cause hydrocephalus in majority of cases, it may remain asymptomatic as in our patient. Prognosis of the condition depends on presence or absence of hydrocephalus.

References

1. Bosemani T, Orman G, Boltshaiser E, Tekes A, Huisman TAGM, Poretti A. Congenital abnormalities of posterior fossa. Radiographics 2015; 35(1): 200-220.
2. Knipe H, Thapa S. Tegmento vermian angle. Radiopedia [Internet]. Available from radiopedia.org/articles/tegmentovermian angle. Accessed on 21st May 2021.

* Associate Professor of Pediatrics
** Resident,
Kilpauk Medical College,
Chennai.
email : childdoctorsri@yahoo.co.in

PICTURE QUIZ

Clinical Background: (Two unrelated kids with with same illness, which is vaccine preventable)

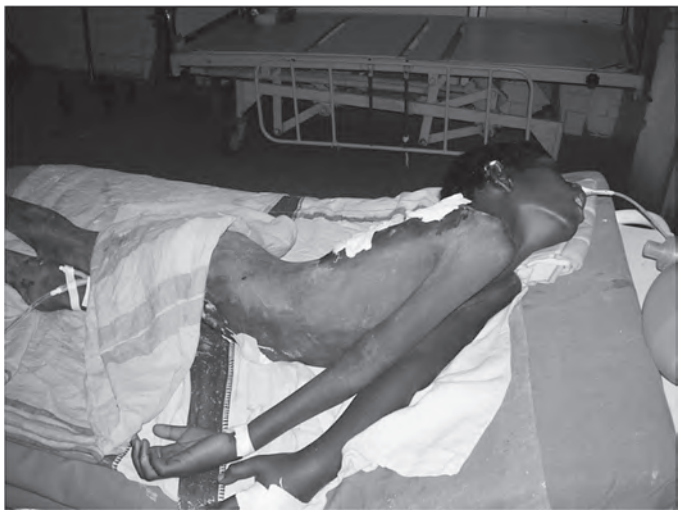


Fig.1. Ten years old presented with CSOM and recurrent seizures. Between the seizures she remained conscious.

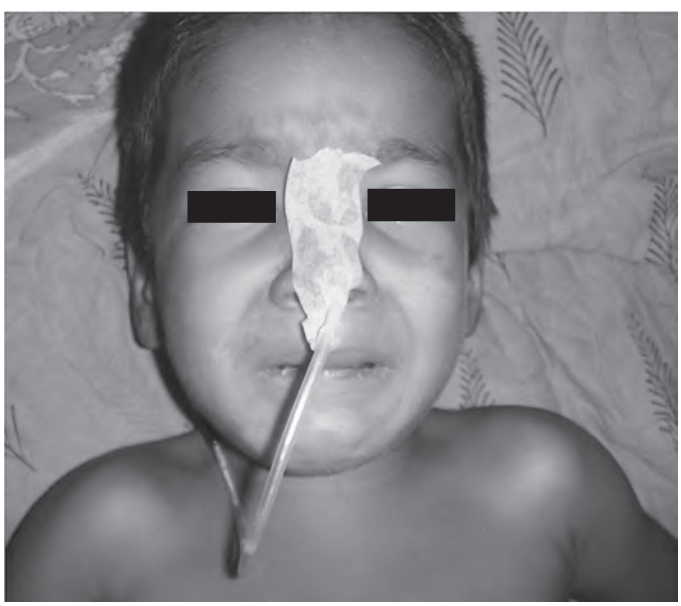


Fig.2a. Four years old boy developed this illness following thorn prick.

Questions:

1. Clinical signs?
2. Diagnosis?

Answers

1. Opisthotonus, Risus sardonicus
2. Tetanus

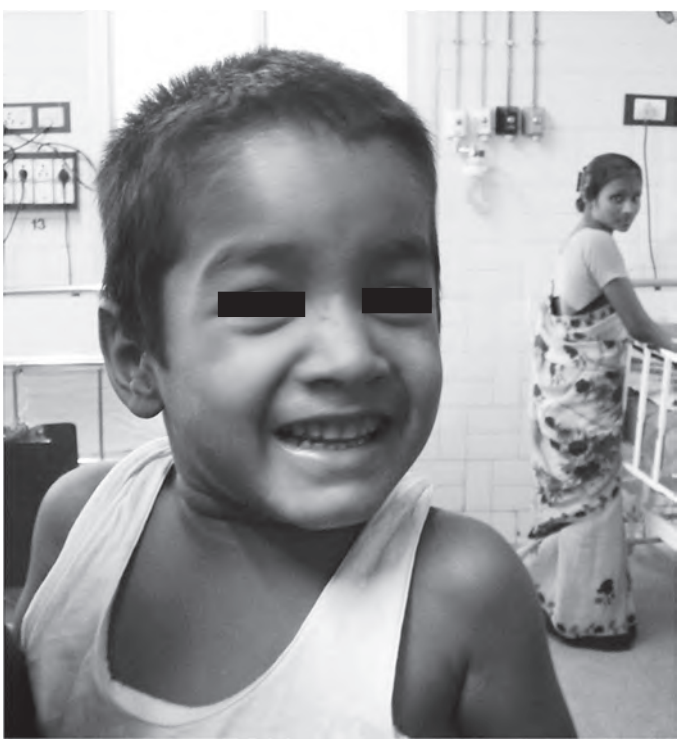


Fig.2b. Two weeks of therapy, recovering.

INDIAN JOURNAL OF PRACTICAL PEDIATRICS

SUBSCRIPTION TARIFF

IJPP

JOURNAL OFFICE
1A, Block II, Krsna Apartments
50, Tamil Salai (Halls Road), Egmore,
Chennai 600 008.
Phone: +91-44-28190032, 42052900.
Email: ijpp_iap@rediffmail.com

Official Journal of the Indian Academy of Pediatrics
A quarterly medical journal committed to practical pediatric problems and management update

For office use
Ref. No.
NEFT / DD / Cheque / Cash
Transaction / DD / Cheque No.
Receipt No. & Date

Enter subscription for

Individual	Institution
ONE YEAR	ONE YEAR
TEN YEARS	FIVE YEARS

(Subscription period is between January and December only)

Name .....

Address .....

.....

City .....State .....

Pin ..... Phone (R) ..... (O) .....

Mobile ..... Email .....

Designation ..... Qualification .....

I am enclosing a DD / cheque No. ..... dated ..... drawn on .....

favoring **Indian Journal of Practical Pediatrics** for Rs.....

Signature

SUBSCRIPTION RATE			BANK ACCOUNT DETAILS (for NEFT)		
Individual	Annual	Rs.750/-	Bank Name	:	THE KARUR VYSYA BANK
	Ten years	Rs.6,500/-	Account Name	:	Indian Journal of Practical Pediatrics
Institution	Annual	Rs.4,000/-	Account Number	:	1154115000004720
	Five years	Rs.16,000/-	IFSC code	:	KVBL0001878
Foreign	Annual	US $ 75/-			

Send your subscription, only by crossed demand draft, drawn in favour of **INDIAN JOURNAL OF PRACTICAL PEDIATRICS,** payable at Chennai and mail to **Dr. S.Thangavelu,** Editor-in-Chief, 1A, Block II, Krsna Apartments, 50, Thamizh Salai (Halls Road), Egmore, Chennai - 600 008. Tamil Nadu, India.

INDIAN JOURNAL OF PRACTICAL PEDIATRICS

ADVERTISEMENT TARIFF

Office Journal of the Indian Academy of Pediatric - A quarterly medical journal committed to practical pediatric problems and management update

Name .....

Address .....

.....

.....

City .....

State ..... **Pin** .....

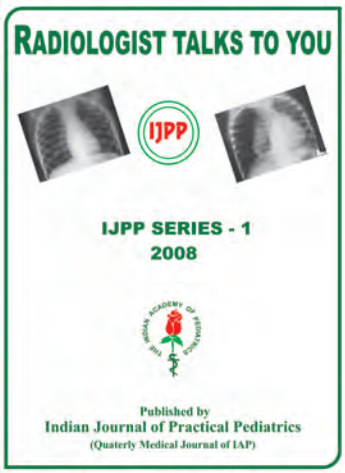
Black & White Advertisement	
Half Page	Rs.5,000.00
Full Page	Rs.10,000.00
Back cover	-
Second cover	-
Third cover	-

Colour Advertisement		
Single Page	Rs.15,000.00	Full Page
Back Cover	Rs.25,000.00	
Second Cover	Rs.20,000.00	
Third Cover	Rs.20,000.00	

Signature

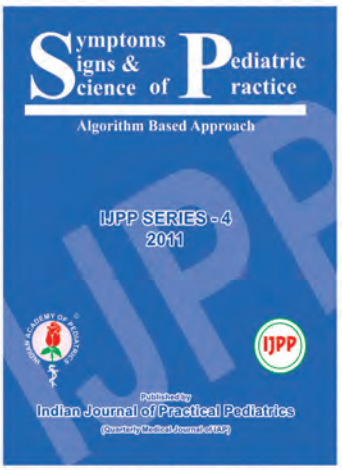
Kindly send your payment by crossed demand draft only, drawn in favour of "Indian Journal of Practical Pediatrics" payable at Chennai.

THE MANAGING EDITOR
Indian Journal of Practical Pediatrics,
1A, Block II, Krsna Apartments, 50, Thamizh Salai (Halls Road),
Egmore, Chennai - 600 008. Tamil Nadu, India.
Tel.No. : 044 - 28190032, 4205 2900
E.mail : ijpp_iap@rediffmail.com



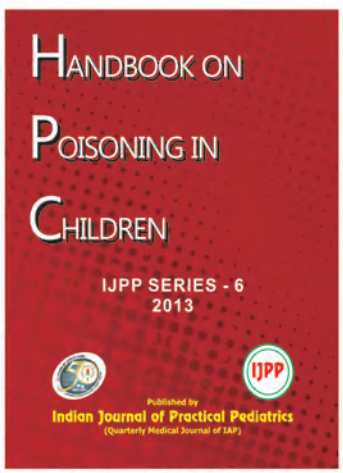
Price : Rs.200/-

Price : Rs.150/-

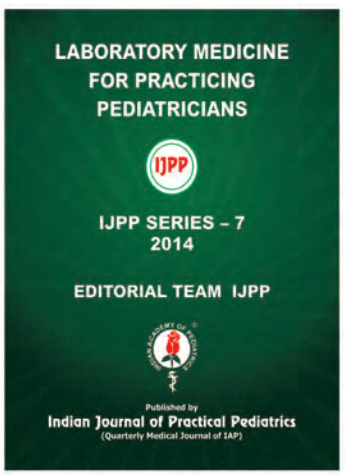


Price : Rs.300/-

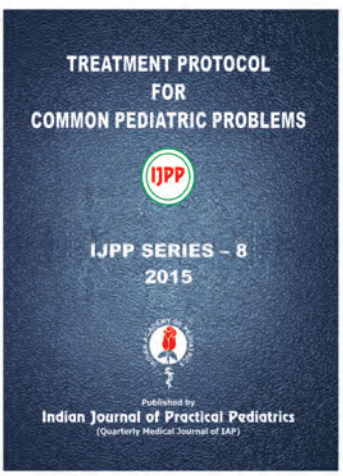
Price : Rs.400/-



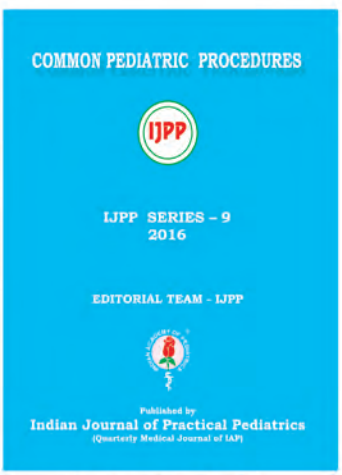
Price : Rs.400/-



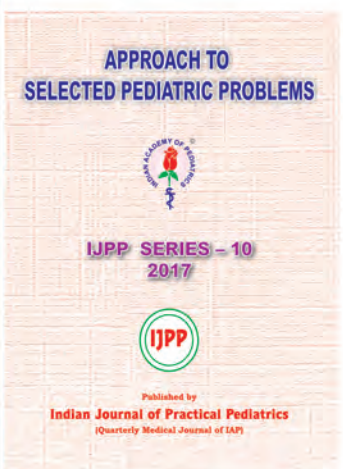
Price : Rs.500/-



Price : Rs.350/-

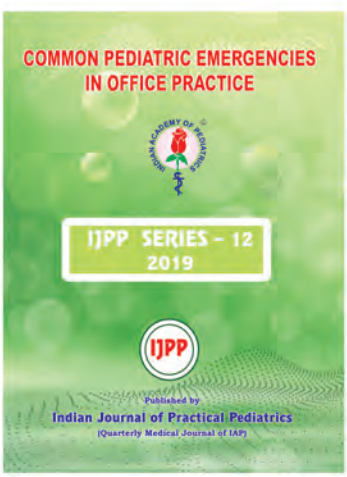


Price : Rs.600/-



Price : Rs.500/-

Price : Rs.650/-



Price : Rs.650/-

Copies available at : Indian Journal of Practical Pediatrics, 1A, Block II, Krsna Apartments, 50, Thamizh Salai (Halls Road), Egmore, Chennai - 600 008. Tamil Nadu, India. Tel.No. : 044 - 28190032, 4205 2900 E.mail : ijpp_iap@rediffmail.com