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ERRORS OF IMMUNITY FOR THE COMMUNITY ALLERGIST: IDENTIFICATION AND INVESTIGATION

Clinical vignette

An 18-year-old male presented with eczema at the age of 6 months. During infancy he also developed multiple food allergies, and progressed with asthma, and allergic rhinitis over time. He had significant dermatitis, frequent acute otitis media, and upper/lower respiratory tract infections often requiring antibiotics. At 3 years of age, he had an Influenza A related pericardial effusion. He was noted to have eosinophilia of 12.5 x 109/L (30%) and elevated IgE (4320 ug/L).

At 4 years of age, he was admitted with a left lobar pneumonia, pansinusitis and was positive for adenovirus. IgE was elevated (9600 ug/L) and he was noted to have persistent eosinophilia 26.9 x 109/L (60%), poor specific antibody response to vaccines, normal levels of IgG, IgA and IgM and normal lymphocyte subsets but with decreased T-cell proliferation. He was diagnosed with combined immunodeficiency and treated with intravenous immunoglobulin (IVIg) replacement and antibiotic prophylaxis.

Over the next few years, he developed eosinophilic dermatitis, esophagitis and pneumonitis, severe asthma, recurrent otitis media and sinusitis, and generalized flat warts. He had dysgammaglobulinemia with low IgA and IgM and high IgE levels (up to 25000 ug/L). Profound lymphopenia developed with very low T-cells; normal B-cell counts but poor antibody responses.

He was ultimately diagnosed with hyper-IgE syndrome secondary to DOCK8 deficiency. While waiting for a matched donor for hematopoietic stem cell transplant (HSCT), he developed squamous cell carcinoma of the skin and died at the age of 21 years. This case highlights how the presentation with common allergic conditions may be early signs of important inborn errors of immunity.

DEFINITION

Human inborn errors of immunity (IEI), referred to as primary immunodeficiency disorders (PID), are a heterogeneous group of disorders, characterized by an increased susceptibility to infection, autoimmune, autoinflammatory, allergic and/or malignant diseases[1]. To date, more than 400 disorders have been genetically identified[2]. Most identified IEI are monogenic variants which result in loss of expression, loss-of-function (LOF; amorphic/hypomorphic), or gain-of-function (GOF; hypermorphic) of the encoded protein[3,4].

PREVALENCE

IEI are common. There is an estimated prevalence of 1 in 1000 to 1 in 5000 live births[7] as illustrated in **Figure 1**[9], with the exception of IgA deficiency (prevalence of 1 in 500 in Caucasians[8]). These conditions may present at any age, although children between the ages of 5 to 19 have the highest prevalence rate (**Figure 2**[10,11]). There is a 1:1 ratio in the gender disposition of IEI per a recent study in the US.

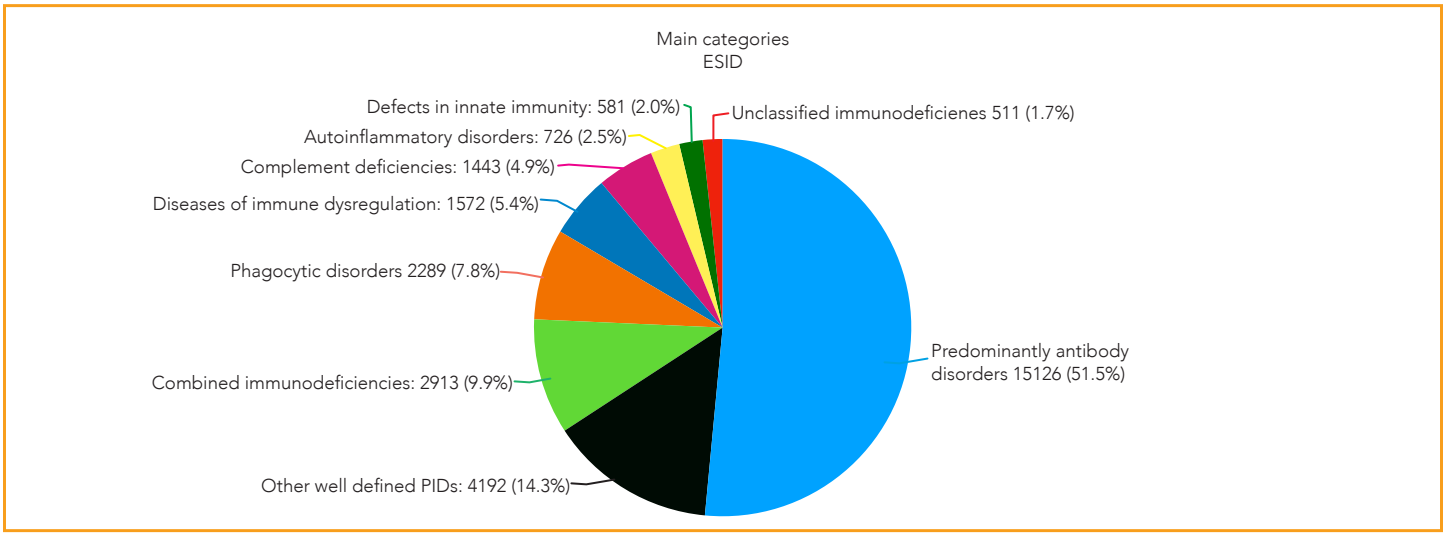

Figure 1. Distribution of major PID groups; Adapted from the Manual of Allergy and Clinical Immunology, 2021

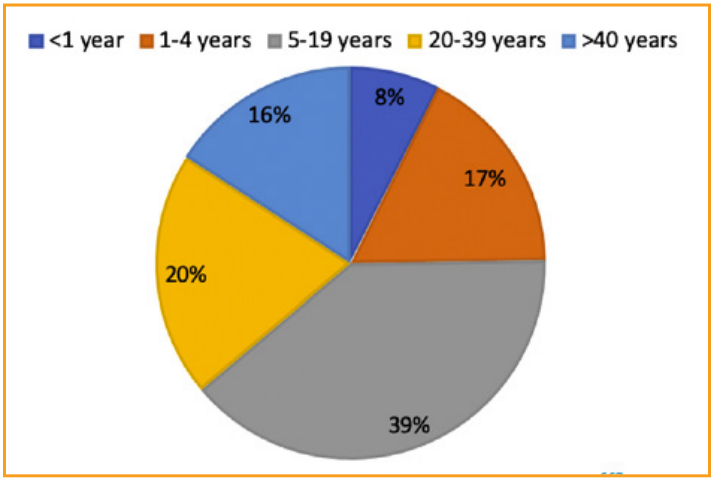

Figure 2. Prevalence of PID by age groups; Adapted from Stiehm's Immune Deficiencies Inborn Errors of Immunity, 2020

CLINICAL APPROACH TO IEI

The immunologic variations of IEI is increasingly complex and a systematic approach for patients with suspected IEI, as described in the questionnaire below[17] is a useful tool for the clinician (**Figure 3**).

Investigations into IEI, should be initiated based on clinical presentations of recurrent infections or evidence of immune dysregulation. Unexplained lymphopenia or persistently abnormal levels of other leukocytes should also prompt investigations. Secondary causes of potential immunodeficiency, including infection, immunosuppressive therapies or malignancy, are also considerations for evaluation of immune function[18,19].

IEI CLASSIFICATION

There are eight categories of IEI per the Expert Committee of the International Union of Immunological Societies (IUIS) (**Table 1**[2,23]). Recently, an IEI classification of two major categories has been proposed: Primary Immune Deficiency Disorders (PIDD) and Primary Immune Regulatory Disorders (PIRD)[24]. Seventy percent of identified disorders are classified as PIDD[25], and are infection dominant conditions. PIRD are dominated by immune-mediated pathologies (autoimmunity, lymphoproliferation, autoinflammation/ hyperinflammation, malignancy and severe atopy)[24]. Defining the precise nature of the PIRD is key to directing clinical management and selecting targeted therapies[26,27].

CLINICAL PRESENTATION

Infections with atypical severity or pathogens, and/or increased frequency are often the first manifestations of IEI. Unusual or early onset autoimmunity, lymphoproliferation or autoinflammation[1] are also suggestive features of IEI. The main categories of IEI, as well as their clinical presentation, are described in **Table 1**[2,23].

IEI may mimic common childhood diseases, including eczematous dermatitis and food allergies. Atopic conditions may result from an underlying immunodeficiency or immune dysregulation[28]. Mechanistically, the development of atopy includes skin barrier disruption, mast cell dysregulation, tolerance failure and impaired T-cell receptor signaling[29]. The classic triad of eczema, elevated serum IgE and eosinophilia are hallmarks of conditions including atopy, hyper-IgE syndromes (HIES), Omenn syndrome and Wiskott-Aldrich syndrome[30]. The differentiation of these conditions in patients with severe atopic dermatitis is still challenging. Findings directing investigations include increased frequency of infection, severity at presentation and comorbidities such as thrombocytopenia. A high index of suspicion is key[31].

Clinical approach to evaluate IEI

1. Is the main concern infection or immune dysregulation?
   - If infection: Are the main infections bacterial, viral, fungal, mycobacterial, or mixed? Obtain information about sites and severity of infection, opportunistic/unusual organisms and therapies required to treat the infections.
   - If immune dysregulation: Are the features autoantibody-driven (i.e., cytopenias, colitis, arthritis), granulomatous or lymphoproliferation?
2. Are there any somatic/dysmorphic features (i.e., short stature, microcephaly, poor wound healing)?
3. Are the exposures unusual?
4. Is there any severe atopy?
5. In children, are there any developmental concerns or reactions to live vaccines?
6. Is there any cause of secondary immunodeficiency (i.e., infections, medications, disease-causing protein loss, malnutrition, hypercatabolic states, chronic illnesses, splenectomy, or malignancy)?
7. Is there a family history of PID, recurrent/severe infection, early/unexplained deaths, autoimmunity, malignancy or autoinflammation? Any history of consanguinity?
8. Are there any clues on physical exam (failure to thrive, thrush, absence of lymph nodes including tonsils, heart/ lung disease, delayed separation of umbilical cord, hepatosplenomegaly, or bone/joint/skin abnormalities)?
9. Are there any laboratory studies already pointing toward a category of deficits or dysregulation?

Figure 3. Questionnaire adapted from Stiehm's Immune Deficiencies, Inborn Errors of Immunity, 2020

Classification and examples	Clinical presentation
1. Immunodeficiences affecting cellular and humoral immunity	
T-B+ severe combined immune deficiency (SCID)	
yc deficiency (common gamma chain SCID, CD132 deficiency)	
JAK3 deficiency	Severe, reccurent opportunistic infections; failure to thrive; diarrhea; rash
T-B- SCID	
RAG 1/2 deficiency	
ADA deficiency	Bone defects, may have pulmonary alveolar proteinosis, cognitive defects
Combined immunodeficiencies (generally less profound than SCID)	
CD40 ligand deficiency	Severe and opportunistic infections, idiopathic neutropenia; hepatitis and cholangitis, Cryptosporidium infections
CD40 deficiency	Neutropenia, opportunistic infections, gastrointestinal and biliary tract and liver disease, Cryptosporidium infections
ICOS deficiency	Recurrent infections, autoimmunity, gastroenteritis, granulomas
2. Combined immunodeficiencies with associated or syndromic features	
Wiskott-Aldrich syndrome	Thrombocytopenia with bleeding and bruising; eczema; recurrent bacterial and viral infections; autoimmune disease
Ataxia telangiectasia	Chronic sinopulmonary disease; cerebellar ataxia; small, dilated blood vessels of the eyes and skin; malignancy
DiGeorge syndrome	Hypoparthyroidism; seizures; cardiac abnormalities; abnormal facies; infection
Hyper IgE syndrome	Chronic dermatitis; recurrent, severe lung infections; skin infections; bone fragility; failure to shed primary teeth
3. Predominantly antibody deficiencies	
XLA	Severe bacterial infections
CVID	Clinical phenotyes vary: recurrent infections, lymphoproliferation, autoimmune cytopenias and/or granulomatous disease
Selective IgA deficiency	Recurrent sinopulmonary infections with encapsulated baceria
Specific antibody deficiency	Autoimmune disease and increased risk of malignancy in CVID
IgG subclass deficiency	
4. Diseases of immune dysregulation	
HLH	Fever, splenomegaly, cytopenia, rash
ALPS	Splenomegaly, adenopathy
IPEX	Autoimmune enteritis, early onset diabetes, thyroiditis, hemolytic anemia, thrombocytopenia, eczema
APECED	Autoimmunity affecting parathyroid, adrenal, other endrocrine organs; candidiasis; dental enamel hypoplasia
5. Congenital defects of phagocyte number or function	
Congenital neutropenias	
Chronic granulomatous disease	Severe infection; abscesses with granuloma formation
Leukocyte adhesion deficiency	Recurrent, severe bacterial infections; poor wound healing; delayed seperation of the umbilical cord
6. Defects in intrinsic and innate immunity	
Mendelian susceptibility to mycobacterial disease (MSMD)	
IL-12 and IL-23 receptor β1 chain deficiency	Susceptibility to mycobacteria and salmonella infections
IFN γ receptor 1 deficiency	
Predisposition to severe viral infection	
STAT 1/2 deficiency	Severe viral infections (disseminated vaccine-strain measles), mycobacterial infection
Herpes simplex encephalitis (HSE)	
TLR3 deficiency	Herpes simplex virus (HSV) 1 encephalitis; severe pulmonary influenza; varicella-zoster virus infection
Predisposition to invasive fungal diseases	
CARD9 deficiency	Invasive candidiasis infection, deep dermatophytosis, other invasive fungal infections
Predisposition to mucocutaneous candidiasis	
STAT1 GOF	CMC, various fungal, bacterial and viral (HSV) infections, auto-immunity (thyroiditis, diabetes, cytopenias), enteropathy
TLR signaling pathway deficiency with bacterial susceptibility	
IRAK4 deficiency	Bacterial infections (pyogens)
Other inborn errors of immunity related to non-hematopoietic tissues	
Isolated congenital asplenia	Bacteremia (encapsulated bacteria)
7. Autoinflammatory disorders	
Type 1 interferonopathies	
STING-associated vasculopathy, infantile onset (SAVI)	Skin vasculopathy, inflammatory lung disease, systemic autoinflammation
Aicardi-Goutieres syndrome	
Defects affecting the inflammasome	
Familial Mediterranean fever (FMF)	Recurrent fever, serositis and inflammation responsive to colchicine
Non-inflammasome-related conditions	
TNF receptor-associated periodic syndrome (TRAPS)	Recurrent fever, serositis, rash, and ocular or joint inflammation
8. Complement deficiencies	
Deficiency in early complement pathway components (C1q, C1r, C2, C4)	SLE-like syndrome, rheumatoid disease, multiple autoimmune diseases, infections
Deficiency in late complement pathway components (C5, C6, C7, C8, C9)	Neisserial infections, SLE-like syndrome
C3 and regulatory components	Recurrent infections with encapsulated bacteria

JAK3 Janus kinase 3, RAG recombination activating gene, ADA adenosine deaminase, ICOS inducible costimulator gene, XLA X-linked agammaglobulinemia, CVID common variable immunodeficiency, HLH hemophagocytic lymphohistiocytosis, ALPS autoimmune lymphapraliferative syndrome, IPEX immunodysregulation polyendocrinopathy enteropathy X-linked, APECED autoimmune polyendocrinopathy candidiasis and ectodernal dystrophy, IL interleukin, JFN interferon-, STAT signal transducer and activator or transcription, TLR Toll-like receptor, CARD9 caspase recuitment domain family 9, GOF gain of function, IRAK4 interleukin 1 receptor associated kinase 4, STING stimulator of interferon genes, CMC chronic mucocutaneous candidiasis, SLE systemic lupus erythematosus

Table 1. Simplify classification of IEI; adapted from the International Union of Immunological Societies: 2019 Expert Committee on IEI

Clinical vignette

The clinical case of early onset of severe atopy with frequent sinopulmonary infections, unusual severe viral illnesses, eosinophilia, and high serum IgE are key elements suggestive of a combined immunodeficiency, particularly hyper-IgE syndromes. HIES is a multi-systemic syndrome characterized by recurrent skin abscesses, pneumonia with pneumatocele formation, eczematous dermatitis, and elevated IgE levels. However, autosomal dominant and autosomal recessive forms of the disease differ significantly in their clinical features, as shown in **Table 2**.

Disease	Genetic Defect	Inheritance	Main Features	Distinguishing features from common allergic disorders
AD-HIES STAT3 deficiency (Job syndrome)	STAT3	AD LOF	Eczema, skin abscesses, CMC, recurrent pneumonias leading to pneumatoceles, and skeletal and connective tissue abnormalities	Early-onset eczema; peculiar thickened texture of the facial skin, retroauricular fissures, and severe folliculitis of the axillae and groin; cold abscesses; distinctive facial and skeletal features, low frequency of allergy
DOCK8 deficiency	DOCK8	AR	Severe eczema, severe allergies, immunodeficiency with increased susceptibility to bacterial and viral infections, autoimmunity, and increased risk for malignancies	Severe eczema associated with warts, severe skin and sinopulmonary infections
PGM3 deficiency	PGM3	AR	Skeletal dysplasia, immunodeficiency and tendency to bone marrow failure, severe atopy, neurodevelopment al delay; some patients display renal, intestinal, and heart defects.	Complex syndromic phenotype associated with atopy
ILG signal transducer (ILGST) deficiency	IL6ST	AR or AD LOF	Largely overlapping with AD-HIES: eczema, recurrent skin and pulmonary infections, craniosynostosis, neurodevelopmental delay	Severe eczema, recurrent cutaneous and pulmonary infections, distinctive skeletal features
Comel-Netherton svndrome	SPINK5	AR	Congenital ichthyosis, bamboo hair, atopic diathesis; increased bacterial infections; enteropathy, failure to thrive	Congenital ichthyosis
TYK2 deficiency	TYK2	AR	Susceptibility to intracellular bacteria (mycobacteria, Salmonella) and viruses; dermatitis	Peculiar susceptibility to infections

Table 2. Clinical features of HIES; Adapted from Castagnoli et al. World Allergy Organization Journal (2021)

INVESTIGATING IEI

Early diagnosis of IEI is critical for prevention of disease-associated morbidity and mortality. This requires a high index of clinical suspicion and early treatment dramatically improves life expectancy and quality of life[1,32].

Laboratory investigations, prompted by the clinical phenotype, are described in **Table 3**. Commonly available tests include complete blood count (CBC) and smear, which may reveal signs of lymphopenia or neutropenia. Additionally, both levels of cells and proteins, as well as their function should be evaluated. For example, a patient may have normal levels of IgG, but additional evaluation of specific antibodies post-vaccination may demonstrate a functional failure (**Table 4**).

Affected immunity arm	Typical site of infection	Common pathogens	Screening tests
B cells/ antiobody	Sinopulmonary tract, GI tract, joints, CNS	Pyogenic bacteria: Streptococci, staphylococci, *Haemophilus influenzae* Enteroviruses: ECHO, polio Mycoplasma	IgG IgA IgM Vaccine responses (titers)
T cells	Sepsis, lung, GI tract, skin	Viruses: CMV, adenovirus, measles, molluscum Fungi: *Candida, Aspergillus, Pneumocystis jirovecii* Protozoa: Cryptosporidium	CBC with differential Flow cytometry for T cells and subsets T cell proliferation to mitogens
NK cells	Skin, lung GI tract, disseminated infections	Viruses: EBV, CMV, VZV, HSV, HPV	Flow cytometry for NK cells CD107a surface expression NK cytotoxicity assays
Phagocytes	Skin infections, lymphadenitis, liver, lung, bone, GI tract, gingivitis/ periodontitis	Bacteria: Staphylococci, *Serratia marcescens, Burkholderia cepacia, Klebsiella, E. coli, Salmonella, Proteus* Fungi: *Candida, Aspergillus, Nocardia*	Neutrophil count DHR Flow cytometry for CD11/CD18
Complement	Systemic infections, meningitis	Pyogenic bacteria: Streptococci, *Haemophilus infuenzae, Neisseria*	CH50

Table 3. Laboratory testing according to the IEI clinical phenotype; courtesy of McCusker, MD and Saker, MD

EVALUATING ANTIBODY RESPONSES

Clinical manifestations such as recurrent sinopulmonary infections and those involving encapsulated bacteria should prompt an evaluation for both primary B cell defects and combined immune disorders[34,35]. Initial screening tests should include a CBC with differential and immunoglobulin quantification such as total IgG, IgA, IgM, and IgE. There are no strict standards for pathologically low immunoglobulin levels, although a serum IgG below 3 g/L in an adolescent or adult and values below the age-matched range in children warrant further evaluation[1]. Albumin levels will rule out protein loss as the underlying cause of hypogammaglobulinemia.

If serum immunoglobulins are detectable, specific antibody response to protein antigens (diphtheria and tetanus vaccines) and polysaccharide antigens (23-valent polysaccharide vaccine) and/or presence of isohemagglutinins should be assessed. Ideally, specific antibody levels are measured pre-immunization and 3 to 4-weeks post-vaccination. Guidelines for normal responses are available to guide clinicians[36]. There are 2 methods defining sufficient response to pneumococcal vaccination. Protection against infection and colonization is associated with antibody concentrations of 1.3 mg/mL or greater. Most patients can mount a 2-4-fold increase over baseline titers post-vaccination if the preimmunization levels are <4. For the diagnosis of immunodeficiency, the general standards of normal responses are at least a 4-fold increase or a level ≥ to 1.3 µg/ml of at least 4 pneumococcal serotypes postimmunization. Vaccine response cannot be reliably evaluated in patients who have received immunoglobulin replacement therapy within the past 4 to 6 months. Other useful tests to evaluate the B cell compartment are listed in **Table 4**.

Assessment	Quantitative	Functional	Advanced Test
Humoral (B-cells)	- Ig levels: IgG, IgA, IgM and IgE - B cell immunophenotyping by flow cytometry (CD19, CD20, CD10, CD21, CD23, CD27, CD38, CD40, CD81, CD138, surface Igs, κ chain, λ chain)	- Natural antibodies (e.g., isohemagglutinins-IgM antibody to blood group A and/or B) - Specific antibody levels Random, pre/post immunization antibody levels to protein (e.g., tetanus toxoid, diphtheria toxoid) and polysaccharide antigens (pneumococcal vaccine)	- IgG subclasses (restricted utility) - Antibody response to vaccination with a neoantigen (Phi X174, rabies, Salmonella typhi) - Class switching - In vitro Ig production (antibody secreting cell generation, ELISPOT for specific Ig production) - Mutation analysis (e.g., BTK, AID, IGHM,...)
Cellular (T-cells)	- CBC with differential - T cell immunophenotyping by flow cytometry (CD3, CD4, CD8, CD45RA/RO, TCR αβ/γδ)	- In vitro lymphocyte proliferation in response to mitogens (PHA, ConA, PWM, PMA+I), CD3/CD28, and antigens (including alloantigens and recall antigens). - TRECs: SCID newborn screening - T-cell receptor Vbeta repertoire (by immunophenotype or spectratyping)	- Extended T cell immunophenotyping (CD3 chains, CD62L, CD31, CCR7, CXCR5, CD40L, CD127, CD132; MHC-I, MHC-II) - In vitro cytokine production - Adenosine deaminase, purine nucleoside phosphorylase levels and % daxP accumulation - Radiosensitivity testing - Mutation analysis (e.g., IL2RG, RAG1/2, DCLRE1C,...)
Phagocytic	- CBC with differential - Morphology: Smear evaluation	- DHR flow cytometry assay (alternative NBT test) for chronic granulomatous disease - Adhesion molecule evaluation: β2 integrins (CD18, CD11a.,b,c), CD15 for leukocyte adhesion defect	- Phagocyte cell i.e. (APC, monocytes) phenotyping (CD14, CD68, CD86, HLA-DR, 7DA, IFNGR1, IL12Rβ1) - Chemotaxis - Bactericidal activity - STAT1/STAT4 phosphorylation in response to IFNγ/IL12 - IL-12 production in response to IFNγ - Mutation analysis (e.g., CYBB, CYBA, NCF1, NCF2, NCF4, IFNGR1, IL12RB1,...)
Complement	C3, C4 C1 esterase inhibitor levels	- Total hemolytic complement (CH50): Classical pathway - Alternative pathway (AH50) - MBL - C1 esterase inhibitor function (for hereditary angioedema)	- Individual complement components - C3 nephritic factor - Mutation analysis (e.g., C1QA, CFB, CFD, MASP2,…)
NK cells	- CBC and differential - NK/NKT cell immunophenotyping by flow cytometry (CD3, CD16, CD56)	- NK cytotoxic activity on K562 cells	- NK cytotoxic activity on other cells (Raji, 721.221, SKBR3) - NK ADCC - NK cytokine production (ELISPOT) - Mutation analysis (e.g., GATA2, IRF8, MCM4, GINS1,...)

Table 4. Laboratory testing to evaluate IEI; courtesy of McCusker, MD and Saker, MD

EVALUATING T CELLS

A history of prolonged viral infections, opportunistic infections, autoimmunity and failure to thrive (in the setting of an affected infant or young child) suggest a possible T cell defect. Initial evaluation includes a CBC focusing on the white blood cell count and absolute lymphocyte count as up to 75% of circulating lymphocytes are T cells. Particularly in infants, lymphopenia may suggest a T cell developmental defect or marked T cell destruction and should prompt immediate immunological evaluation for potentially life-threatening conditions, such as a severe combined immune deficiency (SCID). Low lymphocyte count in isolation, as a screen for SCID, is not adequate as this will fail to identify infants with "leaky (hypomorphic) SCID"[37], who may have normal or even elevated T cell numbers yet profound deficiency in T cell function. Other causes of T cell lymphopenia, including HIV infection or mechanical loss of lymphocytes (e.g., intestinal lymphangiectasia) also should be rapidly ruled out.

NEWBORN SCREENING FOR SEVERE COMBINED IMMUNE DEFICIENCY (SCID)

The evaluation for SCID may be initiated by an abnormal newborn screening test which measures the number of copies of T cell restriction excision circles (TRECs), formed during T cell development[42]. TREC screening alone is not diagnostic for SCID, but requires immediate additional evaluation, including lymphocyte subset immunophenotyping to confirm a failure of T cell development. This is typically followed by lymphocyte proliferation, testing for maternal chimerism, and ultimately genotyping[43].

Importantly, TREC screening identifies classic forms of SCID characterized by <300 T cells/mm^3 at birth, but it fails to capture atypical SCID due to hypomorphic mutations in known SCID genes, as shown in **Table 5**[44].

EVALUATION OF COMPLEMENT SYSTEMS

Specific clinical presentations prompt evaluation of complement defects including encapsulated bacterial infection and angioedema[44]. The complement system is activated by three pathways: the classical, the alternative, and the lectin pathways, all of which converge at C3 to activate a common final pathway (the membrane attack complex). The three complement pathways should be functionally assessed.

Defects of C3 result in susceptibility to encapsulated bacterial infections, whereas defects of C5 to C9 are associated specifically with *Neisseria sp.* infections. C1, C2 or C4 complement deficiency are associated with infection and autoimmunity, such as systemic lupus erythematosus[47]. Functional tests of individual components are available in specialized laboratories (**Table 4**).

EVALUATION OF PHAGOCYTES

Recurrent bacterial and/or fungal infections involving the skin and deep organs are suggestive of a neutrophil defect[44]. Assessment should begin with a CBC for the absolute neutrophil count and a peripheral blood smear for cellular morphology. Several genetic IEI have been identified leading to neutropenia.

Assays of neutrophil function should also be considered. Neutrophil migration to sites of infection and pus production are compromised in leukocyte adhesion deficiency (LAD) and neutrophil killing activity is affected in chronic granulomatous disease (CGD). Rapid screening for CGD can be achieved using the dihydrorhodamine 123 (DHR) assay[52]. Other tests to evaluate the phagocyte compartment are listed in **Table 4**.

EVALUATING NATURAL KILLER (NK) CELLS

Recurrent viral infections and primary hemophagocytic lymphohistiocytosis, suggest a possible NK cell defect[44,53]. NK cell evaluation is done in specialized laboratories (**Table 4**). Classical NK deficiency results when both the number and function of the NK cells are profoundly reduced, whereas, in functional NK deficiency, only the cytotoxic capacity is abnormal in the setting of normal NK-cell counts.

GENETIC TESTING

Access to genetic testing has become an essential and indispensable tool. Gene discovery has accelerated with significant advances including whole exome and whole genome sequencing. As a result, there are currently more than 400 immune disorders genetically identified[2].

The contribution of genetic testing has significant impact on patient care with a shorter time to definitive diagnosis, the identification of asymptomatic family members and better family planning decisions. Genetic testing allows for the identification of molecular defects, allows the use of targeted therapies and increases our understanding of molecular pathways crucial for immune functions.

TREC results	Causes	Absolute T-cell counts and mitogen proliferation testing
TREC critically abnormal or absent/not detectable	Typical SCID or athymic conditions	Absence of or very low CD3 T-cell number (<300/µL) Low to absent naive T cells PHA proliferation <10% lower limit of normal
TREC abnormal or low	Atypical SCID	Low CD3 T-cell number (300-1500/µL) Low to absent naive T cells PHA proliferation 10%-50% lower limit of normal
TREC abnormal or low	Once SCID is rules out, consider other conditions: • Preterm birth • Syndromes associated with T-cell lymphopenia • Secondary T-cell lymphopenia • Idiopathic T-cell lymphopenia	Reduced CD3 T-cell number

NBS, Newborn screen; TREC, T-cell receptor excision circle.
Table 5. Newborn screening for SCID: Test results and possible diagnosis; Adapted from Knight V. et al., 2021

Despite these benefits, the emergence of broad-based sequencing approaches, has also introduced new challenges. The vast amount of genetic information obtained through sequencing, particularly in the form of variants of unknown significance, is problematic due to the need for functional assessments, not always readily available, and the need of genetic counselling.

CONCLUSIONS

Investigation of immune function is essential for accurate diagnoses in patients with recurrent and/or unusual infections as well as those with features of immune dysregulation. Many new diagnostic tools have been added to our medical armamentarium in recent years yet the diagnosis of IEI still relies on the combination of clinical acumen to identify patients at risk, leading to appropriate laboratory and genetic tests. The early evaluation of immune function provides not only critical diagnostic information, but also guides clinical decisions regarding appropriate therapies and prevention of disease-associated morbidity and mortality.

As illustrated in this article and by the clinical vignette, infection may not be the significant presenting feature for IEI. Patients for whom there are clinical suspicions for IEI should be evaluated with screening tests followed by directed protein/cellular and genetic testing. As this remains an evolving field, patients may need to be re-evaluated as our understanding progresses.

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