Assessment of the human immune system course

Department of Internal medicine No.2

Human humoral immunity state assessment

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INTRODUCTION

Inborn errors of immunity (IEI), which is the preferred terminology for primary immunodeficiency disorders, most often come to clinical attention because of an increase in the incidence or severity of infectious illness beyond what is considered "normal." Patients with IEI diseases may also have immune dysregulation that includes unusual or severe autoimmunity, lymphoproliferation, and fevers and severe inflammation that are unprovoked (autoinflammation). Some forms of IEI are also characterized by an increased risk of malignancies.

Intro

Immune dysregulation can result in disorders other than recurrent infections, including:

1. Autoimmune disorders, such as autoimmune hemolytic anemia
2. Inflammatory disorders, such as inflammatory bowel disease or inflammatory arthritis
3. Malignancies, such as lymphoma
4. Allergic disease, such as atopic dermatitis, food allergy, and allergic rhinosinusitis and asthma

Intro

Before initiating immunologic testing, the clinician should perform a thorough clinical history and physical examination. In infants and children, height and weight records should be reviewed as failure to thrive and poor growth are consistent with immunodeficiency. In patients with possible IEI, important historical elements include:

Intro

- The nature of the infections. This should include the frequency, chronicity, severity, and response to therapy. Other considerations include what organ system(s) is involved and what type of organism has been identified in the past (ie, viral, bacterial, fungal, opportunistic). Patterns of infections can suggest specific immune defects.
- Age of onset of illness since different immune problems present in infancy, childhood, and adulthood.
- The patient's sex because X-linked defects are mostly or exclusively seen in boys.
- Family history of infections, abnormal inflammatory responses, and autoimmune disorders, as well as any childhood deaths.
- Family history of lymphoproliferative illnesses, including occurrence of splenectomy.
- Any associated nonimmunologic symptoms and signs, as revealed by a complete review of systems.

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Initial screening laboratory tests — In patients of any age, the laboratory evaluation of the immune system begins with general studies, including

- Complete blood count with differential:

Lymphopenia is characteristic of a variety of combined cellular and antibody deficiency immunodeficiencies.

Lymphopenia is defined as an absolute lymphocyte count <1500 cells/microliter in adults or <2500 cells/microliter in infants.

Neutropenia can be found in primary phagocyte disorders, as well as in neutrophil disorders that lead to secondary immunodeficiency.

Leukocytosis is sometimes noted and suggests chronic infection

Intro

- Chemistry panels to assess for metabolic disorders (diabetes mellitus, renal

disease) that might cause secondary immune deficiency.

Hypoalbuminemia or low serum proteins suggest malnutrition or protein loss. Markedly elevated globulin levels may be seen in gammopathies or chronic infections.

Urinalysis for proteinuria, casts, or cells, which suggest nephritis.

Intro

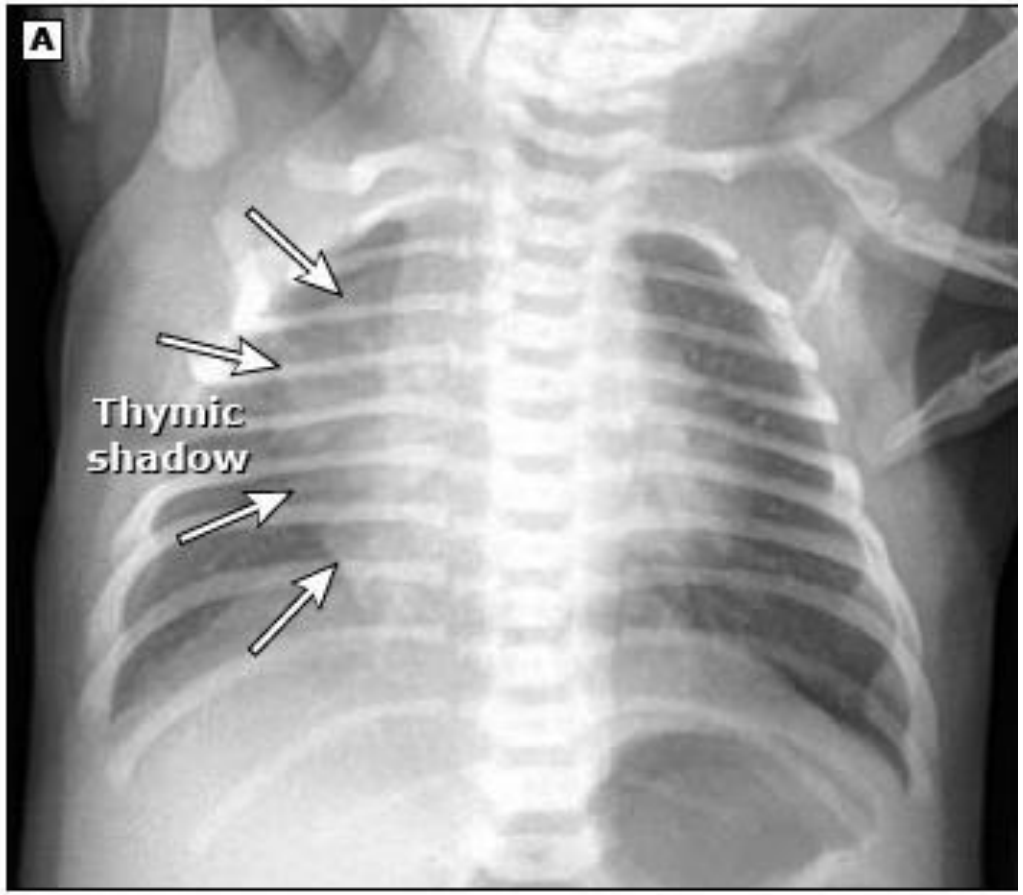
- Tests to evaluate for specific infections, if indicated by the presentation (eg, appropriate cultures, chest and/or sinus imaging):

Sinus films may uncover extensive chronic sinusitis in patients with immune deficiency. Children or adolescents with **nasal polyposis** (although not adults) should be evaluated for cystic fibrosis, which is a cause of frequent sinopulmonary infections.

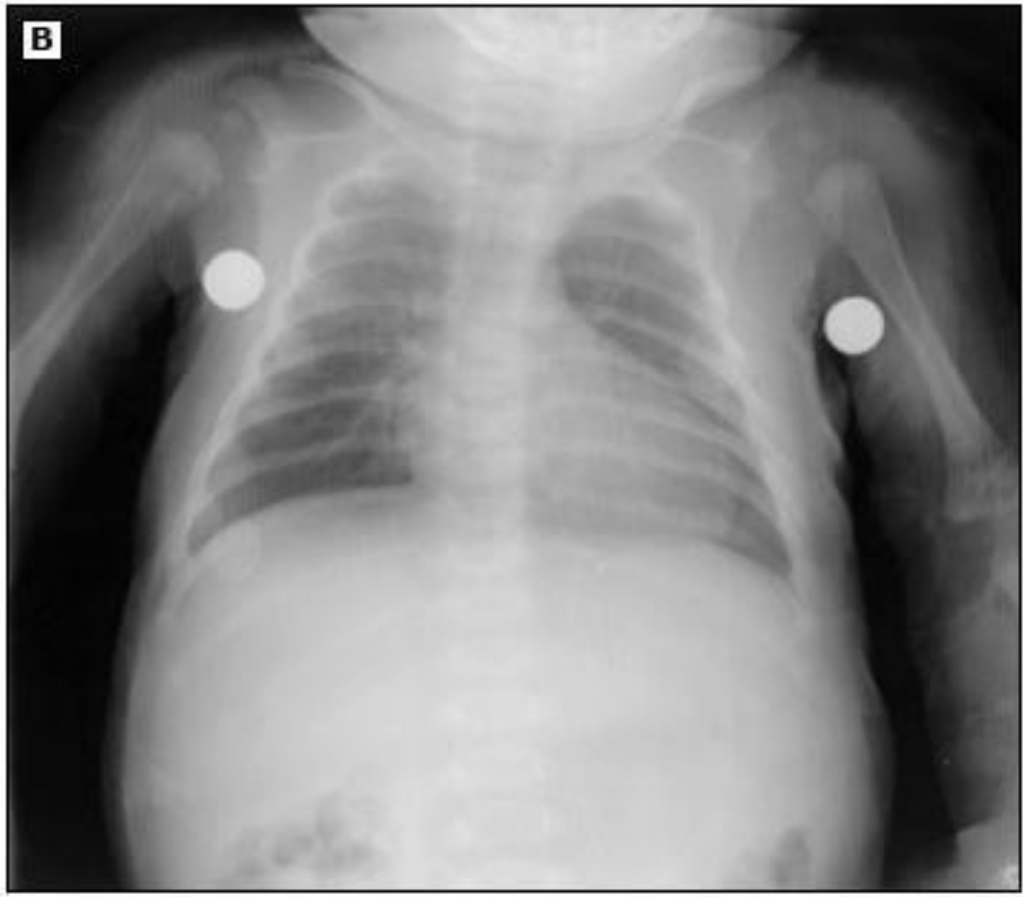
Chest radiographs of an infant showing **absence of a thymic shadow** should prompt an emergent evaluation for severe forms of immunodeficiency.

In older children and adults, chest radiographs may show scarring from past infections, interstitial lung disease, or bronchiectasis.

Hyperinflated lung fields suggest chronic obstructive lung disease or chronic asthma



(A) Chest radiograph showing normal thymic shadow



(B) Chest radiograph showing absence of the thymic shadow in an infant with severe combined immunodeficiency (SCID)

Intro

- **Erythrocyte sedimentation rate and/or C-reactive protein:** Nonspecific elevations in acute phase reactants can be seen with infectious and inflammatory disorders and suggest the need for further evaluation.
- **Referral** — More advanced immunologic tests require varying degrees of expertise to perform and interpret, may not be widely available, and are often costly. In addition, knowledge about the possible diagnoses in question is invaluable in deciding the type of testing to pursue first. Thus, immunologic testing is best performed in a graded fashion, and referral to an allergist/immunologist should besought early in the process, when possible

Intro

The age of the patient can help narrow the differential diagnosis:

The most common antibody defects that present **in infancy** are transient hypogammaglobulinemia of infancy, selective antibody deficiency (after the age of two years) and selective IgA deficiency.

The most common antibody deficiencies **in young children** are selective antibody deficiency, selective IgA deficiency, IgG subclass deficiency, and early-onset common variable immunodeficiency.

The most common disorders that present **in adulthood** include common variable immunodeficiency, selective IgA deficiency, IgG subclass deficiency, and selective antibody deficiency.

Measurement of antibody levels

Measurement of serum immunoglobulin G (IgG), IgA, immunoglobulin M (IgM), and immunoglobulin E (IgE) is useful in all cases of suspected antibody deficiency

There are several methods for determining serum Ig levels, and laboratories use different systems.
Therefore, it is critical that age-adjusted normal reference ranges are provided

Immunoglobulin levels

Serum IgG, IgM, IgA, and IgE levels*				
Age	**IgG (mg/dL)**	**IgM (mg/dL)**	**IgA (mg/dL)**	**IgE (international units/mL)[1]**
Core blood (term)	1121 (636-1606)	13 (6.3-25)	2.3 (1.4-3.6)	0.22 (0.04-1.28)
1 month	503 (251-906)	45 (20-87)	13 (1.3-53)	
6 weeks				0.69 (0.08-6.12)
2 months	365 (206-601)	46 (17-105)	15 (2.8-47)	
3 months	334 (176-581)	49 (24-89)	17 (4.6-46)	0.82 (0.18-3.76)
4 months	343 (196-558)	55 (27-101)	23 (4.4-73)	
5 months	403 (172-814)	62 (33-108)	31 (8.1-84)	
6 months	407 (215-704)	62 (35-102)	25 (8.1-68)	2.68 (0.44-16.3)
7 to 9 months	475 (217-904)	80 (34-126)	36 (11-90)	2.36 (0.76-7.31)
10 to 12 months	594 (294-1069)	82 (41-149)	40 (16-84)	

1 year	679 (345-1213)	93 (43-173)	44 (14-106)	3.49 (0.80-15.2)
2 years	685 (424-1051)	95 (48-168)	47 (14-123)	3.03 (0.31-29.5)
3 years	728 (441-1135)	104 (47-200)	66 (22-159)	1.80 (0.19-16.9)
4 to 5 years	780 (463-1236)	99 (43-196)	68 (25-154)	8.58 (1.07-68.9)¶
6 to 8 years	915 (633-1280)	107 (48-207)	90 (33-202)	12.89 (1.03-161.3)Δ
9 to 10 years	1007 (608-1572)	121 (52-242)	113 (45-236)	23.6 (0.98-570.6)◇
14 years				20.07 (2.06-195.2)
Adult	994 (639-1349)	156 (56-352)	171 (70-312)	13.2 (1.53-114)

IgG: immunoglobulin G; IgM: immunoglobulin M; IgA: immunoglobulin A; IgE: immunoglobulin E.

* Numbers in parentheses are the 95% CIs.

¶ IgE data for 4 years.

Δ IgE data for 7 years.

◇ IgE data for 10 years.

Reference:

1. Kjellman NM, Johansson SG, Roth A. Serum IgE levels in healthy children quantified by a sandwich technique (PRIST). Clin Allergy 1976; 6:51.

Measurement of antibody levels

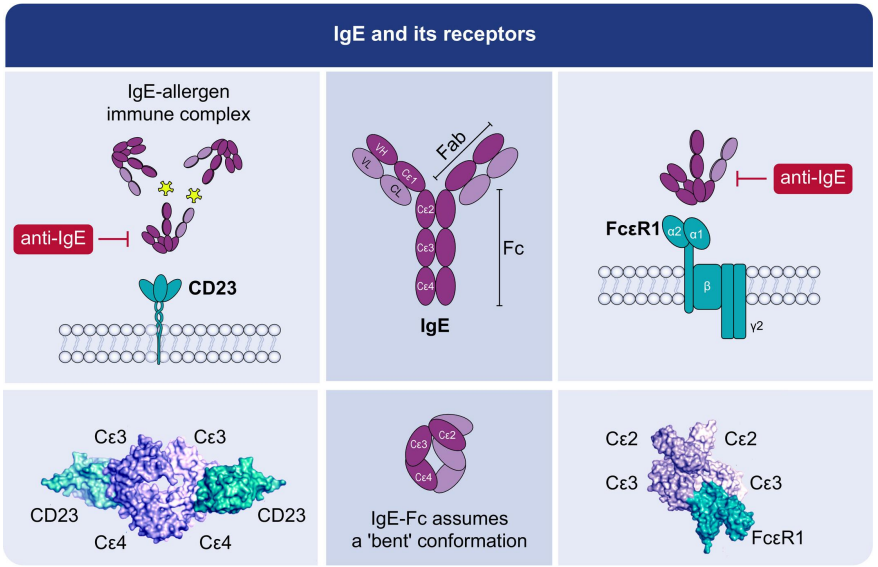
- Hypogammaglobulinemia is defined as an IgG less than two standard deviations from normal, and agammaglobulinemia is usually considered when IgG is <100 mg/dL.
- Panhypogammaglobulinemia is defined as low levels of IgA, IgG, and IgM and is a hallmark of B cell deficiencies, most forms of severe combined immunodeficiency (SCID), and can rarely be seen in certain genetic variants of the common variable immunodeficiency (CVID) phenotype. In combined immunodeficiencies (CIDs), as well as in several predominantly humoral immunodeficiencies, there are characteristic alterations in the profile of immunoglobulin isotypes that may aid in diagnosis (eg, selective IgA deficiency, selective IgM deficiency, and hyper-IgM immunodeficiencies).

An isolated low level of IgA is relatively common and does not necessarily signify an inborn errors of immunity (IEI)

Measurement of IgG subclasses is usually **not** helpful, and it is useless in young children because the expression of IgG subclasses is quite variable in these ages. However, IgG subclasses are sometimes assessed later in the evaluation of antibody defects.

IgE levels can be useful if elevated or low. Elevated IgE levels are helpful in the identification of several monogenic causes of antibody deficiency (eg, signal transducer and activator of transcription 3 [STAT3], interleukin 21 receptor [IL21R], interleukin 6 receptor [IL6R], and interleukin 6 signal transducer [IL6ST] deficiencies, among others). Measurement of IgE is helpful in patients with recurrent sinopulmonary infections or scaly or eczematoid skin disorders as an elevation is consistent with underlying allergic disease (eg, >100 international units/mL). A very elevated IgE (eg, >2000 international units/mL) in a patient with recurrent bacterial or fungal infections and dermatitis would raise suspicion for a hyper-IgE syndrome and several other IEI

Very low or undetectable serum IgE <2 ng/mL is often seen in the CVID phenotype that can be used to distinguish it from other causes of hypogammaglobulinemia



Serum immunoglobulin D (IgD) is not used in the diagnosis of any disorder, though elevated levels of IgD are often seen in one of the rare autoinflammatory disorders (mevalonate kinase [MVK] deficiency).

Measurement of antibody function

Clinically significant impairment in antibody function can be present even when serum antibody levels are normal. Antibody function can be assessed by measuring antibody titers (usually IgG isotype) to specific antigens (also known as specific antibody) in response to intentional immunization or natural infection. There are two major goals to measuring vaccine responses: first, to assess if naïve B cells can respond to a new antigen; second, to assess if memory B cells respond appropriately to an antigen seen in the past. Choosing the right vaccine to test with is critical for properly assessing B cell function.

Antibody function is assessed by examining the patient's response to the two general types of antigens: protein antigens and polysaccharide antigens. Routine vaccinations provide examples of both types:

- **Vaccines that assess responses to protein antigens** - Measurement of antibody titers to tetanus, diphtheria, *H. influenzae* B, and protein-conjugated pneumococcal vaccines (eg, Prevnar) are used to assess response to protein antigens. Antibody titers to other vaccines (eg, hepatitis A and hepatitis B, measles, others) can also be used.
- **Vaccines that assess responses to polysaccharide antigens** - Measurement of antibody titers to multiple serotypes in pneumococcal polysaccharide vaccines (eg, Pneumovax 23) are used to assess response to polysaccharide antigens. This evaluation is useful in adults and children older than two years. This response is particularly important in making a diagnosis of specific antibody deficiency.

If a patient has received immune globulin in the prior six months, measuring vaccine response is difficult because antibodies to most vaccine-associated antigens are abundant in immune globulin preparations. Testing can be achieved if the patient can be vaccinated with a vaccine antigen that is not routinely used such as rabies vaccine (representing a protein antigen) or Salmonella Typhim M, Typhim Vi (a polysaccharide antigen)

Principles of interpretation:

- The combination of low levels of IgG accompanied by low IgA or IgM or both, and poor vaccine responses is a feature of many IEI, including both antibody defects and combined (antibody and cellular) defects.
- Normal responses to immunization (ie, normal antibody function) can occur with subnormal levels of total IgG or of IgG subclasses. This pattern is typically seen with secondary causes of hypogammaglobulinemia, such as that caused by medications, protein loss, or severe malnutrition.
- Impaired vaccine responses in a patient with normal levels of IgG, IgA, and IgM may be evidence of specific antibody deficiency, although medications, malignancies, and some infections can also cause impaired vaccine responses.

Isohemagglutinins are antibodies mostly of the IgM isotype generated in response to polysaccharides of gut flora that cross-react with A or B blood group erythrocyte antigens. They generally appear in the blood by six months of age in individuals who have blood types **other than** AB.

Very low titers in a child suggests poor antibody function. Similarly, antibodies to streptococcal antigens (eg, streptolysin O and anti-DNAase) are normally present in all subjects after two years of age; very low titers also hint at antibody deficiency. However, vaccine responsiveness is generally considered to be a more reliable indicator of intact humoral immune function

IgM antibodies can be assessed by measuring isohemagglutinin titers (anti-A, anti-B). All patients except infants < 6 mo and people with blood type AB have natural antibodies at a titer of ≥ 1:8 (anti-A) or ≥ 1:4 (anti-B). Antibodies to blood groups A and B and to some bacterial polysaccharides are selectively deficient in certain disorders (eg, Wiskott-Aldrich syndrome, complete IgG2 deficiency).

IgG antibody titers can be assessed in immunized patients by measuring antibody titers before and after administration of vaccine antigens (Haemophilus influenzae type B, tetanus, diphtheria, conjugated or nonconjugated pneumococcal, and meningococcal antigens); a less-than-twofold increase in titer at 2 to 3 wk suggests antibody deficiency regardless of Ig levels. Natural antibodies (eg, antistreptolysin O, heterophil antibodies) may also be measured

•Cytokine assays:

•Plasma inflammatory cytokines, such as IL-1, tumor necrosis factor (TNF) alpha, IL-6, and IL-18, are elevated in autoinflammatory disorders and in cytokine storms associated with severe infections, graft-versus-host reactions, and acute flares of primary or secondary HLH. Serial assays may be of value in tracking the therapeutic response. Measurement of these cytokines at baseline and during autoinflammatory "flares" can help identify potential therapies (eg, treatment with anti-IL1 agents).

•Cytokine autoantibodies have been associated with immune disorders including anti-IFN-alpha (associated with severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] susceptibility), anti-IFN-gamma (mycobacterial infection), anti-IL-6 (staphylococcal infections), anti-IL-17A, -17F, and -22 (mucocutaneous candidiasis), and anti-granulocyte macrophage colony-stimulating factor (GM-CSF; pulmonary alveolar proteinosis).

•Cytokine production assays, measured in the supernatants of a patient's cells after culture with various antigens, may be of value in identifying defects in selected patients with severe infections or noninfectious inflammatory disorders, or for monogenic disorders where skewing of T cell cytokine production is affected.

•Single cytokine assays are of limited value in the diagnosis of immunodeficiencies and are mainly used in research.

Tests for complement defects — Complement disorders may be inherited or acquired. Broadly, monogenic complement deficiencies can either incur susceptibility to infection or susceptibility to autoimmunity, with some overlaps. Screening for a classical complement pathway defect is indicated in patients with any of the following:

- Recurrent, unexplained pyogenic infections in whom the white blood count and immunoglobulin levels and specific antibody responses are normal
- Recurrent Neisserial infections at any age
- Multiple family members who have experienced Neisserial infections

In addition, it is reasonable to evaluate the complement system in any patient with systemic lupus erythematosus (SLE) or a familial tendency of SLE. However, this is particularly relevant in those with familial lupus or subacute cutaneous lupus, in whom C1q or C2 deficiency should be excluded

The initial screening test for complement defects is a total hemolytic complement assay (CH50), which assesses classical pathway function and is widely available. If the CH50 is significantly reduced or zero, then the levels of individual complement components are measured. Low levels of multiple components, particularly low C3 and C4, suggest complement consumption rather than a primary complement deficiency. If the CH50 is normal and a complement defect is still suspected, the AH50, a screening test for alternative pathway defects, may be obtained. This test is largely performed in specialized laboratories. Alternative complement deficiencies include properdin and factor D deficiencies, both of which are rare. Each specific complement factor and complement inhibitor can be assessed for its abundance and function in specialized laboratories

Tests for innate immune defects — Defects in innate immune mechanisms should be suspected in patients with chronic mucocutaneous candidiasis, invasive fungal infections, invasive bacterial infections, and severe mycobacterial disease when other immunodeficiencies have been excluded. Many innate immune defects arise in early life (infancy and preschool years) and should be suspected, for example, in a newborn with mycobacterial or fungal sepsis. Many are associated with specific infections (eg, herpes or EBV infections in natural killer cell defects, atypical mycobacterial infection in IL-12/23-IFN-gamma pathway defects, herpes simplex encephalitis in the Toll-like receptor 3 (TLR3) signaling pathway and other severe infections, often associated with poor inflammatory response)

SUMMARY

Inborn errors of immunity (IEI), previously called primary immunodeficiencies, should be considered once the more common causes of recurrent infection have been excluded

Every patient with a clinical phenotype compatible with an IEI should be referred to an immunology specialist early in the evaluation when possible. Immunologic testing is best performed in a graded fashion because many tests require varying degrees of expertise to perform and interpret, may not be widely available, and are often costly

Before initiating immunologic testing, the clinician should perform a thorough clinical history and physical examination. In infants and children, height and weight records should be reviewed to detect failure to thrive and poor growth. Routine laboratories can provide clues about other causes of infections and help focus further immunologic evaluation. Screening laboratories include complete blood count (CBC) with differential, chemistry panels, urinalysis, test to diagnose specific organ involvement (eg, sinus computed tomography [CT] or chest radiograph), and C-reactive protein or erythrocyte sedimentation rate to look for elevations that may accompany chronic infections

Defects in humoral immunity, which account for the majority of IEI, usually result in recurrent and severe sinopulmonary infections. Immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) should be measured initially. The next step in evaluation is the measurement of serum-specific antibody titers (IgG) in response to intentional immunization (vaccine response) or natural infection.

CATEGORIES AND PREVALENCE OF INBORN ERRORS OF IMMUNITY

Inborn errors of immunity (IEI) may be grouped into categories based upon the aspects of the immune system that are predominantly affected and their approximate frequency:

- Predominantly antibody deficiencies (55 percent)
- Immunodeficiencies affecting cellular and humoral immunity (15 percent)
- Congenital defects of phagocyte number or function (10 percent)
- Diseases of immune dysregulation (5 percent)
- Autoinflammatory disorders (5 percent)
- Complement deficiencies (4 percent)
- Combined immunodeficiencies (CIDs) with associated or syndromic features (3 percent)
- Defects in intrinsic and innate immunity (3 percent)
- Bone marrow failure syndromes