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Antibiotic Allergies in Children

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A Practical Guide to Diagnosing Immunodeficiency

Maarja Soomann¹, MD, Austen Worth², MD, PhD,† Dylan Lawless³, PhD,‡ and Johannes Trück⁴, MD, PhD**

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The landscape of inborn errors of immunity (IEI), also known as primary immunodeficiencies, has evolved dramatically over the past decade. The number of confirmed genetic defects underlying IEI has more than doubled, with new defects emerging across all major IEI categories.¹ Many of these disorders manifest as susceptibility to infections, or impaired clearance of pathogens, but infections are only one part of the puzzle. Many IELs instead present with autoimmunity, autoinflammation or lymphoproliferation caused by immune dysregulation, and malignancy can also be an important sign of an underlying IEI. Given the complexity of IEI, no single specialist can have in-depth knowledge of every disorder. However, understanding the fundamental principles of immunodeficiencies and applying a structured diagnostic approach are essential for effective patient evaluation. Diagnostic strategies are typically based on expert consensus, integrating theoretical considerations with clinical experience, while also reflecting the availability of testing modalities. Various guidelines of differing depth and specificity have been developed in Europe^{2,3} and the United States.⁴ In this review, we summarize key diagnostic principles and present a commonly used approach (Fig. 1).

As the spectrum of IEI continues to expand, organizing the available information systematically is essential for maintaining an overview. Since the 1970s, the IEI Committee of the International Union of Immunological Societies (IUIS) has curated and classified known IEI. The latest classification groups these disorders into nine main categories and multiple subcategories based on their underlying mechanisms. It also includes a list of phenocopies of IEI—conditions that mimic IEI but do not arise directly from germline genetic mutations (eg, somatic mutation, autoantibodies mediated disease mimicking IEI). These categories reflect the fundamental components of the immune system: the adaptive immune system, the innate immune system and their shared origins in the bone marrow. IUIS categories 1–3 detail defects of the adaptive immunity, namely combined immunodeficiencies (involving T-cells) and antibody deficiencies (B-cell defects). Defects of the innate immune system are grouped into defects of phagocytes (neutrophils, macrophages and monocytes) (IUIS 5), defects of complement function (IUIS 8) and more generalized defects of the mechanisms by which innate immune cells recognize and respond to pathogens (IUIS 6).

Monogenic predisposition to autoimmune and autoinflammatory disorders, which often involve defects of both acquired and innate immunity are covered in IUIS 4 and 7. Bone marrow function syndromes crucially impact not only on erythropoiesis and thrombopoiesis, but also maintain the continuous supply of immune cells, particularly myeloid cells, natural killer cells and B lymphocytes. These disorders are contained in IUIS 9. Understanding these categories provides a structured framework for navigating the expanding field of IEI. We have used the most recent version of the IUIS IEI classification system to build a web-based version that is also linked with OMIM, HPO terms, ClinVar and other sources and is fully searchable: <https://iei-genetics.github.io>.

In children, infection is the most common presentation of IEI. However, infections are frequent in all children, particularly those under 5 years of age, making it challenging to distinguish physiological from abnormal susceptibility to infection. The Jeffrey Model Foundation's "10 Warning Signs of Primary Immunodeficiency" is a screening tool offering a practical attempt to highlight key indicators of IEI.⁵ Although they have been successful in raising awareness of immunodeficiency, several studies have assessed the evidence base for these warning signs and recommended a reevaluation.⁶ For most IEI, the course of infections and the presence of additional clinical or family history features are more informative than the absolute number of infections alone. For example, a single, persistent respiratory tract infection could be an early indication of a serious immunodeficiency. In contrast, recurrent acute otitis media in an otherwise healthy child with no concerning family history is rarely indicative of an underlying IEI.

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From the *Division of Immunology and the Children's Research Center, University Children's Hospital Zurich, University of Zurich, Zurich, Switzerland; †Department of Immunology and Gene Therapy, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom; and ‡Department of Intensive Care and Neonatology, University Children's Hospital Zürich, University of Zürich, Zurich, Switzerland.

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Address for correspondence: Maarja Soomann, MD, Division of Immunology, University Children's Hospital Zurich, Lengstrasse 30, 8008 Zurich, Switzerland. E-mail: Maarja.Soomann@kispi.uzh.ch.

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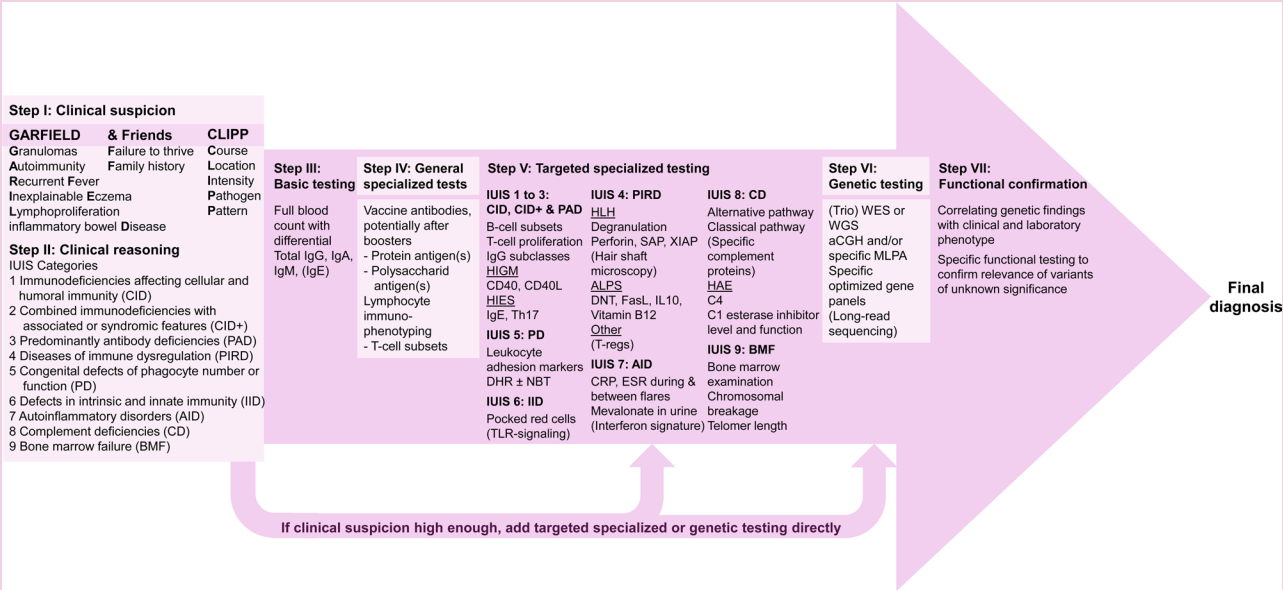


FIGURE 1. Stepwise approach to diagnosing immunodeficiency. ALPS indicates autoimmune lymphoproliferative syndrome; aCGH, comparative genomic hybridization; CD, cluster of differentiation; CRP, C-reactive protein; DNT, double negative T-cells; ESR, erythrocyte sedimentation rate; HIGM, hyper-IgM syndrome; HLH, hemophagocytic lymphohistiocytosis; IL, interleukin; IUIS, International Union of Immunological Societies; MLPA, multiplex ligation-dependent probe amplification; Th, T-helper; TLR, toll-like receptor.

When evaluating for IEI, it is important to consider other factors and comorbidities, which may predispose to infection, independent of immune function. For example, frequent pneumonias may result from primary lung disease (eg, primary ciliary dyskinesia), structural airway anatomy or neurological disorders affecting airway clearance rather than immune dysfunction. Other common comorbidities, which may mimic immunodeficiency include diabetes, severe atopy and congenital cardiac disease.

To improve diagnostic accuracy, alternative approaches to infection assessment have been proposed. German-speaking experts introduced the ELVIS mnemonic,⁷ which can be translated into English as CLIPP to summarize key infection characteristics: course, location, intensity, pathogen, pattern. Beyond infections, immune dysregulation can also indicate IEI. The GARFIELD mnemonic helps clinicians remember critical signs of immune dysregulation: granulomas, autoimmunity, recurrent fever, inexplicable eczema, lymphoproliferation, diarrhea/inflammatory bowel disease. Some of these signs may be obvious; for example, persistent bloody diarrhea in an unwell newborn is highly suspicious and can result from several IELs, including IL10 or IL10 receptor deficiencies. Others, such as lymphoproliferation or autoimmunity, often require active investigation. For instance, patients with splenomegaly and autoimmune

cytopenias due to autoimmune lymphoproliferative syndrome may present with minimal subjective symptoms, as hemolytic anemia may develop gradually, and other cytopenias can remain asymptomatic for extended periods. Finally, failure to thrive and an abnormal family history—including known IEI, a similar phenotype in relatives, consanguinity, or unexplained early deaths—should also raise suspicion of an underlying immunodeficiency. Children identified through newborn screening represent a special case. Depending on the method used in the local program, these may have severe T- or B-cell lymphopenia. In these infants, further diagnostic evaluation must be conducted following local protocols, even in the absence of symptoms or a positive family history. When T-cell lymphopenia is suspected, urgent investigation is required, as the child may have severe combined immunodeficiency (SCID) or congenital athymia.⁸

Once there is reasonable suspicion of an IEI, it is essential to consider which components of the immune system may be affected and the urgency of further evaluation. When choosing the first tests, it is important to keep in mind that there is substantial clinical overlap between different IEI subgroups. For example, frequent or atypical respiratory infections can be caused by various forms of combined immunodeficiencies (IUIS 1 and 2), primarily antibody deficiencies (IUIS 3) or defects of neutrophil number or function

(such as chronic granulomatous disease, CGD) (IUIS 5). It is also essential to remember that most IELs demonstrate marked clinical heterogeneity, and patients often develop infections that are not predicted by the IUIS group into which they fall. For example, a SCID baby may present with a pneumococcal pneumonia rather than the expected *Pneumocystis jirovecii* or viral pneumonitis, and pseudomonal sepsis can be a presentation of X-linked agammaglobulinemia, rather than recurrent respiratory tract infections. As an assessing physician, it is important to be open-minded about the cause of any severe or atypical infection of immune dysregulation.

Basic laboratory tests, such as a full blood count with differential and serum immunoglobulin levels (IgG, IgM, IgA and IgE), are cheap and widely available and can provide valuable initial insights. When interpreted in the context of a detailed clinical history and physical examination, these simple tests can sometimes strongly support the suspicion of an IEI. For example, marked neutrophilia in an infant with delayed cord separation and omphalitis suggests a leukocyte adhesion defect. However, normal results in these tests do not exclude an IEI, and if clinical suspicion remains high, further investigations are warranted. Measurement of antigen-specific—primarily vaccine-specific—antibody responses is particularly informative for diagnosing IEI affecting the adaptive immune system and is often

included in an immunological work-up. However, interpreting vaccine-specific antibodies can be challenging and requires careful consideration of the patient's age, the number of vaccine doses received and the time elapsed since the last dose. For example, the absence of specific IgG against tetanus after completion of the basic vaccination schedule in the first year of life is highly suspicious in a 15-month-old, but may be observed in otherwise healthy children 4 years old and older if booster doses have not yet been administered. A failure to mount a response after a booster remains suspicious later in life, although gradual waning of antibody over time is normal. Due to these and other complexities in distinguishing normal from abnormal vaccine-specific antibody responses in individual patients, these tests are best interpreted by physicians experienced in IEI.

It is important to be aware of several pitfalls in the interpretation of these first line investigations for IEI. All babies have a physiological nadir of IgG levels at between 3 and 9 months of age when maternal IgG wanes before the baby's own IgG production has matured. The timing and severity of this is highly variable making interpretation of IgG levels at this age particularly challenging. Age-appropriate reference values must also be used beyond infancy to avoid over- or underdiagnosis. Certain medications (eg, some anti-epileptic medicines) can lead to secondary hypogammaglobulinemia. In these situations, consultation with a specialist Pediatric Immunologist is advised. Secondary lymph or protein loss can frequently cause lymphopenia or hypogammaglobulinemia despite normal intact immune function.

More detailed immunological investigation typically requires access to a specialized laboratory and expertise in IEI. Given the substantial clinical overlap between different IELs, many experts consider quantification of vaccine-specific antibodies (typically against at least one protein and one polysaccharide antigen, most commonly tetanus toxoid and pneumococcal antigen, and sometimes assessed after booster vaccination) and lymphocyte immunophenotyping with T-cell subset differentiation to be valuable in most patients with suspected IEI. The latter should include, at a minimum, helper T-cells (CD3+CD4+), cytotoxic T-cells (CD3+CD8+) and one or more subsets of naïve T-cells (eg, CD3+CD4+CD45RA+). Unless there is clear suspicion of an IEI not affecting the adaptive immune system, these investigations help exclude the most urgent forms of IEI, such as SCID and certain other forms of combined immunodeficiency. It is also sensible to maintain a low threshold for assessing neutrophil function, as CGD is a more common form of IEI and its presentation can be highly variable. Superoxide

production or oxidative burst assays—most commonly the dihydrorhodamine test, or in some laboratories, the nitroblue tetrazolium test, have high sensitivity and specificity for CGD diagnosis and are widely available.

If the clinical presentation is sufficiently clear to an experienced clinician, selected targeted investigations can be performed in parallel with the above-mentioned broader testing. This is particularly important for hemophagocytic lymphohistiocytosis (HLH), which requires urgent, targeted diagnostics. In addition to the basic work-up required to assess HLH criteria⁹—including an FBC, ferritin, triglycerides, fibrinogen levels and soluble CD25—further specialized immunological tests should be conducted promptly. These include cytotoxic T-cell/natural killer degranulation assays, protein expression analysis of perforin, SAP and XIAP, as well as hair shaft microscopy for primary HLH disorders associated with partial albinism, such as Griscelli syndrome.

When combined immunodeficiencies or primarily antibody deficiencies are suspected, lymphocyte immunophenotyping including T- and B-cell subset differentiation provides valuable additional information. T-cell subsets should include at least helper T-cells, cytotoxic T-cells and naïve T-cells, as outlined above; however, the inclusion of additional markers can further improve diagnostic accuracy. B-cell subset analysis should include, at minimum, naïve and switched memory B-cells (CD19+CD27+IgM–IgD–) as their absence or marked reduction may support the diagnosis of a class-switch recombination defect or a primary antibody deficiency. T-cell proliferation studies may also be useful, though their availability is often limited, and their reliability may be compromised in patients treated with immunosuppression, or who are unwell at the time of testing. For hyper-IgM syndrome, CD40 and CD40L expression following T-cell stimulation can be measured in specialized laboratories, while for hyper-IgE syndromes, assessing Th17 cells may be informative. Diagnosing autoimmune lymphoproliferative syndrome requires specialized testing, particularly quantification of CD4–CD8–TCRαβ+ T-cells (double negative T-cells), along with markers such as Fas-ligand, vitamin B12 and IL10. Complement deficiencies require dedicated functional testing, as they do not typically present with abnormalities in standard laboratory investigations. Simple measurement of C3 and C4 levels may indicate immune activation, but these values are not specific or sufficient to diagnose complement component deficiencies. Instead, functional complement assays that assess the integrity of the classic and alternative pathway should be performed to identify defects in individual components. Asplenia, whether

isolated and congenital due to an IEI, or part of a broader syndromic disorder, can be detected by quantifying pocked red cells in peripheral blood. Functional assays of T-regulatory cell function (CTLA4 recycling), TLR-signaling, mRNA interferon response signature (for type 1 interferopathies), ADA2 activity and quantification of mevalonate in urine (mevalonate kinase deficiency) are all available in highly specialized immunology laboratories, either as an accredited clinical diagnostic test or on a research basis. These investigations demand specialized facilities and well-established workflows, as preanalytical handling issues can lead to false abnormal results. They also require physicians experienced in the interpretation of these results and the clinical presentation of the relevant disorders. There has recently been an expansion in our understanding of cytokine autoantibodies both as part of the pathophysiology of known IELs (eg, APECED) or as phenocopies of immunodeficiency (IUIS 10¹⁰). Detection of these autoantibodies as IEI diagnostic tests is becoming increasingly important and more available.

Alongside the expanding knowledge of IEI, genetic testing technologies have advanced rapidly, enabling broad genomic analyses at reasonable costs. In high-resource countries, the most common approach is trio-based (proband and both biologic parents) whole-exome sequencing (WES) or whole-genome sequencing, often combined with a bioinformatic analysis panel focusing on genes of interest. While these technologies offer significant advantages, it is important to recognize their limitations. Common pitfalls include inadequate sequencing coverage of IKBKG (encoding NEMO, associated with X-linked ectodermal dysplasia and immunodeficiency) and NCF1 (encoding p47^{phox}, deficiency of which leads to autosomal-recessive CGD). These genes are not reliably assessed by standard WES, requiring specialized techniques such as targeted sequencing panels or long-read sequencing. Additionally, copy number variants must be considered, as they may be missed by WES. In such cases, alternative methods are necessary, including comparative genomic hybridization arrays or, in cases of highly specific suspicion, multiplex ligation-dependent probe amplification. The latter is particularly useful for detecting 22q11.2 deletion syndrome or Fanconi anemia.

Ideally, genetic laboratories performing these analyses should have expertise in IEI, as many cases do not yield clearly pathogenic variants in well-established IEI genes but rather variants of unknown significance that may or may not be causative. Assessing the relevance of these variants requires rigorous bioinformatics analysis, complemented

by correlating the clinical and laboratory findings of the affected patients to the existing literature and specialized functional testing in highly specialized immunological diagnostic or research laboratories.

With readily available genetic testing, which can screen for hundreds of IEIs in parallel, the role of extensive specialist immunology testing can feel redundant. However, specialist functional or targeted investigations remain critical in three areas: (1) where there is a high index of suspicion of a specific disorder and a targeted investigation will give a much more rapid result than waiting for genetic results, (2) for confirmation of genetic variants where pathogenicity is not certain, (3) where genetic testing is negative. It is crucial to remember that many adults presenting with IEIs and many children with IEIs will continue to have genetically undefined or unexplained immunodeficiencies, and functional assays are required to characterize these disorders and provide guidance for prognosis and future treatment.

Although genetic testing has become an invaluable tool, it will not replace

clinical reasoning and functional testing in the foreseeable future. A solid understanding of immune system functioning, and the skillful application of quantitative and functional immunological assays remain essential tools in the immunologist's diagnostic toolkit.

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