ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Hierarchy in the diagnosis of inborn errors of immunity and the decline of functional diagnosis

Arq Asma Alerg Imunol. 2024;8(4):429-30.
http://dx.doi.org/10.5935/2526-5393.20240058

Dear Editor,

The immune system is a complex network of cells, tissues, and molecules that protects the body against infections and diseases. Its effective action is due to the diversity of its components, the interconnectivity between them, and the precise regulation of inflammatory and immunological response. In addition, immunity interacts with the microbiome and is influenced by external factors, such as exposure to pathogens and the environment. However, mutations in genes involved in immune response can compromise of this system's functioning, resulting in vulnerability to infections, immune dysregulation, and predisposition to neoplasia, a set of conditions known as inborn errors of immunity (IEI)[1].

These errors encompass several diseases and immune deficiencies and require a complex diagnostic approach. IEI are diagnosed at 3 levels: clinical, functional, and genetic. Despite technological advances, clinical diagnosis remains essential. It is the first step in identifying immunological conditions, allowing syndromes to be suspected based on symptoms, clinical signs, and susceptibility to infection. It is essential to direct subsequent laboratory investigations by grouping conditions into categories such as phagocyte disorders, defects in humoral immunity, cellular immunity, complement system, immune regulation, autoinflammatory disorders, predisposition to specific infections, etc. Although clinical diagnosis does not accurately identify the specific disease, it is crucial to the investigation process and to determine more specialized tests. Only after clinical diagnosis is it possible to define the functional tests necessary to clarify the pathological process and to direct genetic research[2].

Functional diagnostics complement clinical assessment by providing objective data on immune system performance, demonstrating its response to pathogens or stimuli. Tests such as antibody dosage, lymphocyte phenotyping, and lymphoproliferation tests assess the immune response by verifying the production of antibodies and the activation of T, B and NK cells. In addition, phagocytosis disorders, such as chronic granulomatous disease and leukocyte adhesion deficiency, are investigated through tests such as nitroblue tetrazolium and dihydrorhodamine, which assess phagocyte capacity to produce reactive oxygen species to eliminate microorganisms and the expression of adhesion molecules. The CH50 and AH50 tests measure the functionality of the classical and alternative complement pathways, helping identify immunological defects. These tests are essential for identifying immune system dysfunction and guiding treatment.

Much of the syndromic diagnosis of IEI is performed through a combination of clinical and functional diagnoses[3]. Despite the importance of these tests in IEI diagnosis, their availability is limited. The evaluation of humoral immunodeficiencies is more common, with many laboratories offering assays to assess antibody levels and function, although analyzing cellular immunity, the complement system, innate immunity, and phagocytosis is challenging due to the scarcity of specialized laboratories, the high cost, and the low demand. Thus, combining clinical and functional diagnosis, although essential, often becomes unfeasible in certain conditions.

Genetic assessment of IEI is essential to identify immune system mutations, using techniques such as whole-exome sequencing, whole-genome sequencing, and gene panel sequencing. Its main advantages include accurate molecular diagnoses, which are essential in rare diseases, enabling genetic counseling and personalized treatment, such as molecularly targeted therapies, bone marrow transplantation, and gene therapy. However, challenges include the high cost of testing and limited access to specialized centers, in addition to the difficulty of interpreting the results, which requires extremely complex computational analyses. The analysis of genome and whole-exome tests is based on bioinformatics and mathematical tools to interpret huge volumes of genetic

data. The process includes aligning the DNA sequence with a reference genome, identifying variants, and filtering and classifying relevant variants. Predictive tools assist in the interpretation of variants of uncertain significance, and machine learning models identify genetic patterns. These data are integrated with clinical information to obtain more accurate diagnoses and guide treatment[4].

Frequent identification of genetic variants of uncertain significance complicates clinical decisions, since these variants do not have a clearly defined impact on health. The time needed to obtain results can be long, delaying diagnosis and treatment. Furthermore, environmental and epigenetic factors, which influence disease progression, are not considered in genetic testing. The psychological impact on patients and families, especially in relation to hereditary risk, can be significant, reinforcing the importance of appropriate genetic counseling[5].

Interpreting genetic results in IEI is complex, since not all identified mutations are clinically relevant. Many genetic variations do not cause pathology, resulting in the classification of several genetic alterations as variants of uncertain significance. The designation 'variant of uncertain significance' generates uncertainty about whether the variant is benign or pathogenic, making therapeutic decisions difficult. Reclassifying variants of uncertain significance depends on new scientific evidence, especially in rare diseases such as IEI, for which data are limited. Collaborative databases are essential to improve diagnosis and facilitate more effective decisions. Although genetic diagnosis has become more accessible, functional assays are in decline, becoming less available and less requested by clinicians. Genetic evaluation is often preferred over functional evaluation, especially to investigate processes such as phagocytosis and cellular immunity. However, a lack of functional assay data can result in bias and misdiagnosis, especially when relying solely on genetic diagnosis. This is compounded by the inaccuracy associated with variants of uncertain significance, which can lead to incorrect diagnosis[6].

Without functional evidence demonstrating immune dysfunction, IEI diagnosis may be vulnerable to bias. Integrating functional and genetic testing is essential for a complete and accurate assessment of immune conditions. Although genetic diagnosis is a powerful tool, it is important not to overestimate it, since it does not solve all diagnostic challenges. Genetic analysis, while vital, is not a substitute for functional assays, which are crucial to identify true immune system dysfunction.

References

1. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014 Mar 27;157(1):121-41.
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 Jan;40(1):66-81.
3. Richardson AM, Moyer AM, Hasadsri L, Abraham RS. Diagnostic Tools for Inborn Errors of Human Immunity (Primary Immunodeficiencies and Immune Dysregulatory Diseases). Curr Allergy Asthma Rep. 2018 Feb 22;18(3):19.
4. Boycott KM, Vanstone MR, Bulman DE, MacKenzie AE. Rare-disease genetics in the era of next-generation sequencing: discovery to translation. Nature Reviews Genetics. 2013;14:681-91.
5. Johnston JJ, Biesecker LG. Databases of genomic variation and phenotypes: existing resources and future needs. Hum Mol Genet. 2013;15,22(R1):R27-31.
6. Boycott KM, Rath A, Chong JX, Hartley T, Alkuraya FS, Baynam G, et al. International Cooperation to Enable the Diagnosis of All Rare Genetic Diseases. Am J Hum Genet. 2017;100(5):695-705.

No conflicts of interest declared concerning the publication of this letter.

Maurício Domingues-Ferreira
Dewton de Moraes Vasconcelos
Dalton Luis Bertolini
Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Ambulatório das Manifestações Cutâneas dos Erros Inatos da Imunidade do Departamento da Dermatologia do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo - São Paulo, SP, Brazil.