Flow Cytometric Evaluation of Primary Immunodeficiencies

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KEYWORDS

- Immunodeficiency • Flow cytometry • T-cell defect • B-cell defect
- Immunophenotyping

KEY POINTS

- Flow cytometric procedures allow the detection of abnormalities in peripheral blood of primary immunodeficiency patients.
- In the first step, immunophenotyping of B^-, NK^-, $CD4^+$, and $CD8^+$ cells, and HLA-DR/CD38 analysis is recommended to differentiate between normal activation and abnormalities in lymphocyte subsets.
- In a second step, a further differentiation of T, B, and/or NK cell subsets is necessary, for example, in common variable immunodeficiency or DiGeorge syndrome.
- As a third step, functional tests are indispensable in many immunodeficiencies, for example, chronic granulomatous disease or hyper immunoglobulin M syndrome.

FLOW CYTOMETRY AND IMMUNODEFICIENCIES

The term immunodeficiency describes the insufficient immune response to potentially harmful antigens. In general, immunodeficiencies can be divided into primary immunodeficiency diseases (PID) and secondary immunodeficiency diseases.

Primary Immunodeficiency Diseases

PIDs are genetic disorders that mostly cause susceptibility to infections and are sometimes associated with autoimmune and malignant diseases.[1] Mutations can affect cells and molecules of the innate (chronic granulomatous disease [CGD]; complement deficiencies; leukocyte adhesion defects) as well as the adaptive immune system (T and/or B cells). Further, PIDs are part of complex inherited

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syndromes. In the Online Mendelian Inheritance in Man (OMIM) database,[2] hundreds of mutations causing immunodeficiencies or diseases associated with immune dysfunctions have been described. In the United States, the incidence of PID is estimated to be around 1:1200.[3] The first signs of an immune dysfunction can be found in children, often in the early childhood, but also in adults; this depends on the underlying defect.[4] In general, the clinical representation can be dominated by infections, tumors, chronic inflammation,[5] or even signs of autoimmunity[6] or allergy,[7] but there are also patients without any symptoms. This difference is caused by immune dysfunctions inducing simultaneously deficient and autoreactive actions.[8,9] Despite clinical diversity, severe and recurrent infections remain the cardinal signs of immunodeficiencies.[10]

According to the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee of the World Health Organization, PID can be classified into the following groups[11]:

1. Immunodeficiencies affecting cellular and humoral immunity;
2. Combined immunodeficiency disease with associated syndromic features;
3. Predominant antibody deficiencies (recurrent bacterial infections);
4. Diseases of immune dysregulation;
5. Congenital defects of phagocyte number, function; or both;
6. Defects in intrinsic and innate immunity;
7. Autoinflammatory disorders;
8. Complement deficiencies; and
9. Phenocopies of PID.

First, a patient suspicious for a primary immunodeficiency should be evaluated by a thorough clinical and family history as along with a physical examination. Owing to the heterogeneity of clinical symptoms caused by immunodeficiencies, clinical awareness remains crucial.[10,12] Specific therapeutic options remain limited to immunoglobulin replacement therapy and stem cell transfer. First approaches with gene therapy and reports about targeted therapies with biologicals are rare. In most cases, treatment is limited to symptomatic therapy as well as the prevention of complications. The first pass laboratory tests include complete blood cell count followed by the testing of more specific immune parameters, including quantitative serum immunoglobulin levels and specific antibody determination.[13,14]

The most appropriate and encompassing screening of the cellular immune system is accomplished by flow cytometry (FCM) for specific subset markers or indicators for functional abnormalities.[15] Today, FCM allows parallel detection of multiple parameters in many individual cells.[16,17] Immunophenotyping of PID provides diagnostic clues for classifying patients and predicting clinical outcome. In addition, the evaluation of intracellular proteins associated with selected PIDs has been proven as a useful diagnostic method.

As a first step, a general cellular overview with simple immunophenotyping of B cells, natural killer (NK), $CD4^+$, $CD8^+$, and double-negative T cells and analysis of HLA-DR/CD38 is recommended to differentiate between normal activation and severe deficiencies of lymphocyte subsets. For example, the general cellular overview is useful to detect the loss of B cells in Brutons disease; $CD4^+$ and $CD8^+$ deficiencies in DiGeorge syndrome (DGS); T, B, and/or NK cell deficiencies in SCID; or greater numbers of double-negative T cells in autoimmune lymphoproliferative syndrome (ALPS). Alternatively, for severe combined immunodeficiencies with absence of T and/or B cells, newborn screening assays are promising[18]; however, these tests commonly miss immunodeficiencies without profound cytopenia.