Advances in *in vitro* diagnostics in allergy, asthma, and immunology in 2012

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Laboratory tests play an increasing role in risk assessment, diagnostics, and disease monitoring. Great advances have been achieved lately, particularly in the field of clinical immunology and allergy. These include neonatal screening of immunodeficiencies and asthma biomarkers and investigation into the role of recombinant allergens in *in vitro* testing. The latter area has implications for the diagnostics of food allergy, pollen-induced allergies, asthma, and insect allergies. (J Allergy Clin Immunol 2013;132:1287-92.)

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Abbreviations used
CCD: Carbohydrate determinant
CVID: Common variable immunodeficiency
FENO: Fraction of exhaled nitric oxide
GCR: Glucocorticoid receptor
SCID: Severe combined immunodeficiency
SLIT: Sublingual immunotherapy
TREC: T-cell receptor excision circle
WDEIA: Wheat-dependent, exercise-induced anaphylaxis

Laboratory tests play an increasing role as routine diagnostic procedures in both inpatient and outpatient services. The increasing knowledge about the mechanism and pathogenesis of diseases not only results in the development of novel therapeutic strategies but also in the discovery of novel biomarkers. With advent of personalized medicine, which is now on the horizon for allergy and asthma treatment, biomarkers are needed to guide therapeutic decisions. However, these biomarkers require careful clinical evaluation, and they need to be benchmarked against currently available gold standards.

The better understanding of disease mechanisms goes hand in hand with advancements in key technologies, which have been originally developed for research purposes and are now increasingly available for routine diagnostic procedures. Important examples include the following: flow cytometry, array- and chip-based diagnostic tools, mass spectrometry, and the development of recombinant tools. These techniques allow a broader and more detailed diagnostic assessment of the molecular and cellular aspects of immune functions. Important examples represent recombinant allergens for the assessment of allergen-specific sensitization.

However, laboratory tests play an important role in more than just the establishment of clinical diagnoses. Increasingly, *in vitro* tests for risk assessment and stratification of patients are becoming available, leading to a personalized approach. Furthermore, diagnostic tests play an increasing role in assessing pharmacologic responsiveness or nonresponsiveness. Another important area is the use of laboratory tests in the identification of life-threatening severe diseases even before clinical onset of symptoms. This is the goal of neonatal screening, which has now also emerged in our field of clinical immunology.

In this article important advances in this area will be described that have been published in the *Journal of Allergy and Clinical Immunology* throughout the year 2012.

IMMUNODEFICIENCIES

Population-based neonatal screening has now arrived in the field of immunodeficiency diseases. Severe combined immunodeficiency (SCID) comprises a group of severe diseases affecting the immune system. Infants with SCID are healthy at birth but die of severe infections unless they receive hematopoietic stem cell transplantation, enzyme replacement therapy, or gene therapy. SCID is characterized by severe deficiencies in T- and B-cell function and also, in some types of the disease, natural killer cell function. This is a genetic disease, with mutations detected in more than 13 different genes thus far.[1] Among several methods that have been considered for SCID screening, quantitative assessment of T-cell receptor excision circles (TRECs) has been successfully validated.[2] TRECs are formed during recombination of the T-cell receptor α genes. During this process, DNA fragments are excised that are not further incorporated into the mature T-cell receptor locus, and they join at their ends to form DNA byproducts termed T-cell receptors. They are stable but do not replicate during cell division, resulting in a dilution of these byproducts throughout T-cell proliferation. Therefore TRECs

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are biomarkers for newly produced T cells, indicating a normal program of T-cell development.

Quantitative PCR assays have been developed to measure these byproducts. The DNA can be extracted from dried blood spots and collected on the well-known and widely used Guthrie card. This is already used for the screening of important metabolic defects. Starting with the US state of Wisconsin,[3,4] almost 1 million TREC screening assays have been performed as of May 2011, detecting 14 cases of SCID, 6 cases of SCID variants, and 40 infants with various forms of T-cell lymphopenia.[5] Recently, the first case of SCID identified in the course of such a pilot program in the state of Massachusetts has been published in this journal.[6] Most states use homebrewed modifications of the TREC assay,[7-10] with the exception of California, which is using a TREC assay under development by PerkinElmer (Waltham, Mass). The available data indicate that these assays fulfill the requirements for population-based screening because false-negative results, or the failure to identify true cases, is kept to an absolute minimum. This is to the expense of a small number of false-positive results, which require further follow-up. Thus far, the tests have proved very sensitive but not completely specific.[2]

Further follow-up of positive screening results include qualitative and quantitative assessment of lymphocytes and lymphocyte/T-cell subsets. However, the age-adjusted reference range in the newborn period has to be taken into consideration.[11] As an alternative for the DNA-based TREC assay, a method based on tandem mass spectrometry has been also proposed.[12] This assay was developed to detect adenosine deaminase deficiency, which is one cause of SCID (an autosomal recessive form), leading to the accumulation of toxic metabolites in the purine salvage pathway. These metabolites are detected by using this method. The assay was validated in a pilot population-based screening program in Tuscany, Italy, and compiled 4 dried blood-spot samples from patients with genetically confirmed adenosine deaminase–deficient SCID and more than 12 thousand samples from healthy newborns. Recently, several guidelines for population-based[8] and patient-centered[13] have been proposed.

The most common symptomatic primary immunodeficiency is common variable immunodeficiency (CVID). The disease is most frequently diagnosed in adults age 20 to 40 years, and there still is a 6- to 8-year delay in establishing the diagnosis in many patients. CVID is associated with a number of chronic complications, including autoimmunity, chronic lung disease, inflammatory bowel disease, systemic granulomatosis, and lymphoid hyperplasia malignancy. A genetic cause of CVID is in most cases unknown, and polymorphisms have been described in a number of genes. A recent article highlights the laboratory diagnosis in these patients.[14] Reduced levels of total IgG, IgA, and/or IgM and a demonstrated deficiency in specific antibody production are essential criteria. The total IgG level is generally less than 400 mg/dL, and if IgG levels decrease to less than 200 mg/dL, initiation of immunoglobulin replacement therapy is strongly recommended.

The qualitative assessment of antibody function is of great importance in the diagnostic work-up of primary immunodeficiencies. Diagnostic vaccination and measurements of the specific antibody responses against such standardized antigens represents a well-established routine procedure. A recently published document by the American Academy of Allergy, Asthma & Immunology Working Group of the Basic Clinical Immunology Interest Section[15] provides a state-of-the-art overview on this procedure and interpretation of the results. Despite a frequent use of diagnostic vaccination, the authors conclude that there is a need for further studies to firmly establish a normal range of antibody titers in response to vaccine antigens, particularly to such antigens with which there is a variable response. Furthermore, they point out that in the end, the clinical status of the patient dictates the therapeutic intervention and not solely the test result.

Administration of standardized immunoglobulin preparations represents an important therapeutic approach to various antibody deficiency diseases. As a relatively rare but nevertheless severe side effect, anaphylactic reactions to immunoglobulin infusions have been reported, and they were attributed in patients with undetectable IgA to the presence of IgG or IgE antibodies directed against IgA. Reports of reactions to immunoglobulin products in IgA-deficient patients have been recently reviewed.[16] On the basis of a thorough examination of these cases, the authors conclude that IgG therapy should never be withheld from an IgA-deficient patient solely because of concerns about the risk of rare adverse events. Furthermore, they conclude that there are insufficient data to warrant a general recommendation for screening for anti-IgA antibodies in these patients because the predictive value of a positive result is still not established. Subcutaneous infusions appear to be tolerated by patients with anti-IgA antibodies.

A recent review and evaluation of a patient with hyper-IgM syndrome[17] pointed to the immunologic phenotype of this condition. For a number of different reasons, IgM B cells have a defect in class-switch recombination, which could be due to defects in cell-cell interaction, intrinsic defects in B-cell development, or DNA repair mechanism. Importantly, the authors point out that patients present with low IgG, IgA, and IgE levels together with either normal or increased IgM levels. Because IgM levels are not necessarily high, they claim the term hyper-IgM as a misnomer.

ALLERGY AND ASTHMA

Asthma biomarkers

The area of personalized and stratified therapeutic approaches has now also arrived in the field of asthma therapy. A small but considerable number of asthmatic patients have poorly controlled disease with standard therapy. Several recently published clinical trials illustrate that such patients might benefit from treatment with biological agents targeting cytokines of the T_H2 immunobiology (eg, IL-5 and IL-13) if they present with an immunomolecular phenotype of high T_H2 responses.[18,19] Therefore there is the need for the development of blood tests to accurately predict treatment responses in such patients.[20]

Periostin represents a biomolecule that has recently received major attention in this regard. It has been shown that periostin promotes eosinophilic inflammation in response to T_H2 cytokines.[21] However, upregulation of periostin is not restricted to this type of inflammation but is rather considered a tissue response to injury and stress. Periostin is expressed in the epithelium of the respiratory tract and gut. Its levels are increased in patients with certain cancers, fibrosis, pulmonary hypertension, and dermatitis.[22-25]

A set of biomarkers was recently evaluated in 67 asthmatic patients who remained symptomatic despite maximal inhaled corticosteroid treatment.[26] The aim of the study was to identify systemic biomarkers of eosinophilic airway inflammation, which