



Laboratory Tests

CHAPTER

16

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Laboratory Evaluation of the Immune System

Laboratory studies are essential to evaluate the immune system to determine the presence of primary immunodeficiency disease. The laboratory evaluation of a person's immune system is usually prompted by an individual experiencing some clinical problems such as a recurrent and/or chronic infection. Information regarding the types of organisms, the sites of infection and the therapies required to effectively treat the individual's infection

often help focus the laboratory studies. It is critical to recognize that it is the patient's medical history and physical exam that direct the appropriate choice of laboratory tests.

This chapter will focus on basic approaches in using the laboratory, limitations in using this data and a general concept of how to interpret laboratory data.

Normal Versus Abnormal Laboratory Values

An important aspect of interpreting any laboratory value, especially those relating to the immune system, is what values are considered normal and what values are considered abnormal. To determine what is "normal," samples are obtained from a group of healthy individuals, who most often are adults and equally divided between males and females.

Once the test is given to these "normal" individuals, the results can be used to determine what the "normal" range is for these tests using a variety of statistical approaches or tools. One of the more common statistical measurements is called a 95% confidence interval, which is a calculated range that includes 95% of the "normal" results. Other statistical approaches that can be used include calculating a mean and standard deviation for the results from the "normal" individuals. In all of these calculations, the tested "normal" group is viewed as representing the general "normal" population. The range generated from the "normal" group can be used to decide if a result from a patient is "normal" or "not normal." It is important to note that by definition when the "normal range" is set to include a 95% confidence interval, 5% of the remaining samples from "normal" individuals are outside this (normal) range; 2.5% will have values above the range and 2.5% will have values below the range.

Using the measurement of height as an example, normal individuals can be just above or just below a normal range (or 95% confidence interval) and still be normal. Someone 1 inch taller than the 95% confidence interval is not necessarily a giant and someone 1 inch shorter is not necessarily a dwarf. In fact, by definition, 2.5% of normal individuals will fall below the 95% confidence limit and 2.5% will fall above!

The fact that 5% of otherwise normal healthy individuals will fall outside the normal range is important when looking at laboratory results—finding a value outside of the reference range does not automatically represent an abnormality. The clinical relevance of an "abnormal" laboratory finding must be based on the clinical history as well as the size of the difference from the normal range.

Another important issue to consider for the proper interpretation of laboratory results is that the data must be compared to the appropriate normal group or reference range. This is a crucial issue for tests of immune function related to age because the immune system undergoes substantial development during childhood. The range of test values that are "normal" in infancy will probably be quite different when the child is 2 or 20 years old. Consequently, all studies in children must be compared to age-related reference ranges. If the laboratory reporting test results does not provide age specific information, it is important to consult with a specialist who can suggest appropriate age-specific reference ranges. Optimally, this should be provided by the laboratory performing the tests, but if these are not available, it is acceptable to interpret laboratory results using published age-specific reference ranges.

The actual laboratory tests chosen should be based on clinical history and physical examination. The laboratory work-up can be broken up into approaches used to evaluate immune disorders characterized as antibody deficiencies, cellular (T-cell) defects, neutrophil disorders and complement deficiencies.

These four major categories of tests for immune deficiencies are described below. Information about evaluative approaches, tests used to screen for abnormalities and more sophisticated testing used to better characterize the disorder are included.

Major Categories of Tests

Laboratory Evaluation For Antibody Deficiency

The standard screening tests for antibody deficiency are measurement of quantitative immunoglobulin levels in the blood serum. These consist of IgG, IgA, and IgM levels. The results must be compared to age-specific reference ranges, taking into consideration the substantial changes in immunoglobulin levels during infancy and childhood.

There are also tests for specific antibody production. These tests measure how well the immunoglobulins that are present in the blood serum function as antibodies aimed at specific antigens such as bacteria and viruses. In this approach, the fact that the patient has been immunized with common vaccines, including those that have antigens made of proteins (e.g. tetanus toxoid, diphtheria toxoid) and those with carbohydrate antigens (e.g. Pneumovax, Hib vaccine) is used to see how well the patient is able to form specific antibodies against these various types of antigens. In some instances, the patient may have already been immunized with these vaccines as part of their normal care, while in other instances the patient may need to be immunized or re-immunized with the intent of examining their response after a specific time interval. The use of the two different types of vaccines is necessary because certain patients with recurrent infections (and normal or near normal quantitative immunoglobulin levels) have been identified with an abnormality in the response to carbohydrate antigens, but a normal response to protein antigens. It is worth noting that during the maturing of the immune system, the response to carbohydrate antigen vaccines lags behind the response to protein antigen vaccines. The interpretation of vaccine responses is best done by a physician who deals with immunodeficient patients on a regular basis.

The ability to evaluate the antibody response in a patient receiving immunoglobulin replacement is far more difficult. This is because immunoglobulin is rich in most of the specific antibodies that are generated following immunizations. When immunized with common vaccines, it is difficult to tell the difference between the antibody provided by the immunoglobulin treatment and any that might have been made by the patient. The solution to this is to immunize with vaccines that are not normally encountered by the general population and therefore are unlikely to be present

in immunoglobulin preparations. Uncommon vaccines, such as typhoid or rabies vaccine, can provide these new antigens. It is important to note that in a patient with a previously confirmed defect in antibody production, stopping therapy to recheck for antibody levels and immunization response is unnecessary and may place the patient at risk of acquiring an infection during the period when the treatment is stopped.

Additional studies used to evaluate patients with antibody deficiencies include measuring the different types of lymphocytes in the blood by staining those cells with chemicals that can identify the different types of cells. A commonly used test is called flow cytometry that can identify B-cells present in the circulation (e.g. CD19 and CD20 positive cells). The B-cell is the lymphocyte that has the ability to become the antibody factory. Certain immune disorders associated with antibody deficiency have an absence of B-cells (e.g. X-linked agammaglobulinemia) as a characteristic feature.

In addition, analysis of DNA for mutations that are associated with a particular disease can be used to confirm a particular diagnosis (e.g. the gene encoding Bruton tyrosine kinase [BTK] associated with X-linked agammaglobulinemia). Finally, there are studies done in specialized laboratories that involve culture systems to assess immunoglobulin production in response to a variety of different stimuli.

Evaluation of Cellular (T-Cell) Immunity

The laboratory evaluation of cellular or T-cell immunity focuses on determining the number of T-cells and evaluating the function of these cells.

The simplest test to evaluate possible decreased or absent T-cells is a complete blood count (CBC) and differential to establish the total blood (absolute) lymphocyte count. This is a reasonable method to access for diminished T-cell numbers, since normally about three-quarters of the circulating lymphocytes are T-cells and a reduction in T-lymphocytes will usually cause a reduction in the total number of lymphocytes, or total lymphocyte count. This can be confirmed by using flow cytometry with reagents that identify either the entire population of T-cells (e.g. CD3) or subpopulations of T-cells (e.g. CD4 and CD8 cells).

The measurement of the number of T-cells is often accompanied by cell culture studies that evaluate

Major Categories of Tests continued

the functional capacity of T-cells. Most frequently this is done by measuring the ability of the T-cells to respond to different types of stimuli including mitogens (e.g. phytohemagglutinin [PHA]) and antigens (e.g. tetanus toxoid, candida antigen). The T-cell response to stimuli can be measured by observing whether the T-cells begin to divide and grow (called proliferation) and/or whether they produce various chemical mediators called cytokines (e.g. gamma interferon). There are an increasing variety of functional tests that are available to evaluate T-cell function, all aimed at providing data that allows quantitative assessment of T-cell functional status. However, it remains somewhat difficult to interpret the diagnostic significance of T-cell functional data that falls in between the extremes of markedly diminished and entirely normal function.

Many of the primary cellular deficiencies are associated with genetic defects. This is particularly true with severe combined immune deficiency (SCID) where more than 10 different genetic causes for SCID have been identified. These can all be evaluated using current technology for mutation analysis and this approach provides the most accurate means to establish the definitive diagnosis.

Evaluation of Neutrophil Immunity

The laboratory evaluation of the neutrophil begins by obtaining a series of white blood cell counts (WBC) with differentials. The WBC and differentials will determine if there is a decline in the absolute neutrophil count (neutropenia). This is the most common abnormal laboratory finding with a clinical history that suggests defective neutrophil immunity. More than one WBC is necessary to rule out cyclic neutropenia.

A careful review of the blood smear is important to rule out certain diseases that are associated with abnormalities in the structure of the neutrophil, or the way it looks under the microscope. An elevated IgE level may also suggest the diagnosis of Job's (hyper IgE) syndrome. If these initial screening tests of neutrophil numbers are normal, testing would then focus on two possible primary immune disorders: chronic granulomatous disease (CGD) and leukocyte adhesion deficiency (LAD). Both of these disorders have normal or elevated numbers of neutrophils and each of these disorders has distinctive features that can help to direct the appropriate evaluation.

Laboratory testing to diagnose CGD relies on the evaluation of a critical function of neutrophils that kills certain bacteria and fungi—the creation of reactive oxygen. This leads to a process called the oxidative burst that can be measured using a number of different methods including a simple dye reduction test called the Nitroblue Tetrazolium (NBT) test. In addition, a more recent testing approach uses flow cytometry to measure the oxidative burst of activated neutrophils that have been preloaded with a specific dye (dihyrorhodamine 123 or DHR) referred to as the DHR test. There is a third evaluation used by some laboratories called a chemiluminescence test. The DHR test has been used for more than ten years, and it is extremely sensitive in making the diagnosis. As a result of its excellent performance, this test has become the standard in most laboratories supporting clinics that see CGD patients regularly. The best confirmation of the specific type of CGD is suggested by the results of the DHR test, but requires confirmation by either specifically evaluating for protein involved or its related gene mutation underlying the disease.

Laboratory testing for the most common form of LAD Type 1 involves flow cytometry testing to determine the presence of a specific protein on the surface of neutrophils (and other leukocytes). This protein is part of a set of surface receptors that form the Beta-2 integrins, proteins that are necessary for normal neutrophil motility or movement. When absent or significantly decreased, the movement of neutrophils to sites of infection is hampered and produces a large increase in the number of these cells in the circulation.

Laboratory Evaluation of Complement

The finest screening test for deficiencies in the complement system is the total hemolytic complement assay or CH50. In situations with a defect in one complement component, the CH50 will be almost completely absent. Specialized complement laboratories can provide additional testing that will identify the specific complement component that is defective. There are some extremely rare conditions in which there are defects in another complement pathway. These can be screened for by using a functional test directed specifically at this pathway, the AP50 test.

Summary

Laboratory testing plays a central role in the evaluation of the immune system. All results must be compared to appropriate reference ranges to avoid misinterpretation. An accurate medical history, family history and physical examination are critical in developing the best strategy for laboratory evaluation, and the orderly use of laboratory testing is strongly recommended. This typically begins with screening tests, followed by more sophisticated (and costly) tests that are chosen based on the initial test results. The range of laboratory testing available to evaluate the immune system continues to expand. This has been driven in part by the recognition of new clinical syndromes associated with recurrent and or chronic infections. It is the direct link between the clinical findings and laboratory testing that has extended our understanding of immune deficiency diseases. The continuation of this trend and laboratory testing of the future will likely be even more sophisticated and help provide further answers to the underlying basis of the expanding range of primary immunodeficiencies.