Laboratory Tests

Chapter 21

Laboratory studies are necessary to determine the presence of a primary immunodeficiency disease. This is usually prompted by an individual experiencing some clinical problems, particularly recurrent and/or chronic infections. Information regarding the types of organisms, the sites of infection and the therapies required to treat the infections often help focus the laboratory studies. The patient's medical history and physical exam direct the appropriate choice of laboratory tests.

Normal vs. Abnormal Laboratory Values

An important aspect in the proper interpretation of any laboratory value is what values are considered normal or abnormal. To determine what is normal, samples are obtained from a group of healthy individuals, usually adults and equally divided between males and females. These results are used to determine what the normal range is, using a variety of statistical approaches. A common statistical measurement is called a 95% confidence interval, which is the range that includes 95% of the normal results. Another statistical test often used is to calculate the mean (the average) and the standard deviation of the mean. One standard deviation above and below the mean includes 65% of the values and 2 SDs encompass 95% of the values. Thus, values that deviate more than 2 SDs represent 2.5% that are unusually high or 2.5% that are unusually low. It is important to note that when the definition of the normal range is set as a 95% confidence interval, the 5% of the selected normal population outside the 95% will fall in the abnormal range, even though they were originally selected as being normal. This is one of the challenges with using statistical methods to define a normal range and must be remembered when evaluating a test result falling near either end of the normal 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 little person. In fact, by definition, 2.5% of normal individuals will be below the 95% confidence limit and 2.5% will be 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 is the group that was used to determine the normal range. This is crucial since the immune system undergoes substantial development during infancy and 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 with age-matched controls. If the laboratory reporting test results does not provide age specific information, it is important to consult with a specialist who knows the age-specific reference ranges. Optimally, the laboratory doing the test should provide this, but if unavailable, there are published age-specific reference ranges.

The laboratory tests used to evaluate immune disorders are used to identify antibody deficiencies, cellular (T-cell) defects, neutrophil disorders and complement deficiencies. These four major categories of tests for immune deficiencies are described on following pages.

Laboratory Evaluation for Antibody Deficiency, or Humoral Immunity

The standard screening tests for antibody deficiency starts with measurement of immunoglobulin levels in the blood serum. These consist of IgG, IgA and IgM levels. The results must be compared to age-matched controls.

There are also tests for specific antibody production. These tests measure how well the immune system responds to vaccines. In this approach, the patient is immunized with common vaccines, including those that have protein antigens (such as tetanus toxoid, diphtheria toxoid) and those with carbohydrate antigens (such as Pneumovax, HiB vaccine). Blood samples are obtained immediately prior to and approximately four weeks after the immunization to evaluate how well the patient forms specific antibodies.

In some instances, the patient may have already been immunized with these vaccines as part of their normal care and will already have circulating antibodies (if they make antibodies), while in other instances the patient may have little or no specific antibody prior to the immunization. The use of different types of vaccines is necessary because certain patients with recurrent infections (and normal or near normal 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 maturation 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 patients with primary immunodeficiency diseases on a regular basis.

The ability to evaluate the antibody response in a patient already receiving immunoglobulin replacement therapy is 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 serve this purpose.

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 replacement therapy is stopped. However, in a patient whose diagnosis of a humoral immunodeficiency is unclear, it may be necessary to stop replacement therapy for a period of four to six months so that the patient's humoral immunity can be adequately assessed.

Additional studies used to evaluate patients with antibody deficiencies include measuring the different types of lymphocytes in the blood by marking those cells with molecules that can identify the different types. A commonly used test is called flow cytometry that can identify B-cells (and other kinds of lymphocytes) present in the circulation. The B-cell is the lymphocyte that has the ability to produce antibody. B-cells may be absent in certain immune disorders associated with antibody (such as X-linked Agammaglobulinemia [XLA]).

In addition, analysis of DNA can be used to confirm a particular diagnosis (such as the gene encoding Bruton tyrosine kinase [BTK] associated with XLA.) Finally, there are studies done in specialized laboratories to assess immunoglobulin production by cultured lymphocytes in response to a variety of different kinds of stimuli.

Evaluation of Cellular (T-Cell) Immunity

The laboratory evaluation of cellular or T-cell immunity focuses on determining the numbers of different types 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 markers specific for different types of T-cells.

The measurement of the number of T-cells is often accompanied by cell culture studies that evaluate T-cell function. This is done by measuring the ability of the T-cells to respond to different types of stimuli including mitogens (such as phytohemaglutinin [PHA]) and antigens (such as tetanus toxoid, candida antigen). The T-cell response to these various stimuli can be measured by observing whether the T-cells divide and grow (called proliferation) and/or whether they produce various chemicals called cytokines (such as interferon). There are an increasing variety of functional tests that are available to evaluate T-cells. An immunologist is the best person to undertake this interpretation.

Many immune deficiencies are associated with specific genetic defects. This is particularly true of Severe Combined Immune Deficiency (SCID) where more than 12 different genetic causes for SCID have been identified. These can all be evaluated using current technology for mutation analysis, and this is the most accurate means to establish the definitive diagnosis.

Evaluation of Neutrophil Function

The laboratory evaluation of the neutrophil begins by obtaining a series of white blood cell counts (WBC) with differentials. The WBC and differential will determine if there is a decline in the absolute neutrophil count (neutropenia). This is the most common abnormal laboratory finding when a patient presents with a clinical history that suggests defective neutrophil immunity. Usually more than a single CBC and differential is necessary to diagnose neutrophil problems.

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 Syndrome (Hyper IgE Syndrome) along with other clinical features that are associated with this syndrome. If these initial screening tests of neutrophil numbers were 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 process, called the oxidative burst, can be measured using a number of different methods including a simple dye reduction test called the Nitroblue

(Evaluation of Neutrophil Function continued)

Tetrazolium (NBT) test. A more recently developed test uses flow cytometry to measure the oxidative burst of activated neutrophils using a specific dye (dihydrorhodamine 123 or DHR), referred to as the DHR test. The DHR test has been used for more than 15 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 patients with CGD 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 the defective 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). When this protein is 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 as well as an increased susceptibility to bacterial skin, oral and other infections.

Laboratory Evaluation of Complement

The standard 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 negative. 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 (the “alternate”) complement pathway. These can be screened for by using a functional test directed specifically at this pathway, the AH50 test. The complement cascade can also be initiated by the mannan-binding pathway and there are some patients with a deficiency in mannan binding lectin.

Laboratory Tests of Innate Immunity

Laboratory tests are also available to measure the function of the various elements of innate immunity. This includes determining the number and activity of lymphocytes such as natural killer cells, as well as the function of various cell surface receptors such as the toll-like receptors.

Looking to the Future

Newborn screening for severe T-cell immunodeficiency is now recommended by the Secretary of the Department of Health and Human Services and has become a reality in more than 10 states, at time of publication, with more to follow. Newborn screening should make the successful cure of SCID and other related severe T-cell immunodeficiencies easier since infants with these conditions will be identified at birth and appropriate treatment, such as immune reconstitution using bone marrow (hematopoietic stem cell) transplantation, can be readily undertaken. *(See chapter titled "Newborn Screening.")*

Genetic testing (mutation analysis) is likely to undergo significant changes in the near future based on the newer technologies. This enables genetic evaluation of large parts of or the entire genetic code for an individual at relatively low cost. These types of approaches are referred to in discussions of personalized medicine based on an individual's unique genetic code, but when this will become reality at a clinical level remains to be defined.

Summary of Laboratory Tests

Laboratory testing plays a central role in the evaluation of the immune system. All results must be compared to age-appropriate reference ranges. An accurate medical history, family history and physical examination are critical in developing the best strategy for laboratory evaluation. This typically begins with screening tests, followed by more sophisticated (and costly) tests 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 primary immunodeficiency 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.