Current Report — HIV

Laboratory Testing for the Presence of HIV Infection and the Progression of HIV Disease

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Laboratory tests for human immunodeficiency virus (HIV) infection are commonly performed for two reasons. First, diagnostic tests can determine whether a person has been infected by the virus. This testing is critically important for patient counselling.[1] At times, diagnostic testing may help establish whether infections, malignancies, or conditions such as weight loss or neurologic dysfunction are due to HIV infection. Second, certain tests have been used as "prognostic indicators" to estimate the stage and activity of HIV disease. These tests have also become important in identifying patients who might benefit from prophylactic therapy against HIV and *Pneumocystis carinii*.[2]

Tests to Establish the Diagnosis of HIV Infection

The diagnosis of HIV infection can be made by specific antibody tests, antigen assays, the polymerase chain reaction, and viral culture.

The principal HIV antibody tests are the *enzyme-linked immunosorbent assay* (ELISA) antibody test and the *Western blot* test. Antibody testing should be performed by experienced laboratories using a combination of tests that is both sensitive and specific. The ELISA test is quite sensitive, with false-negative tests occurring less than 3 percent of the time. Because of its sensitivity, the ELISA test is useful for initial screening. Like most screening tests, however, false-positive tests will occur, necessitating further specific testing. The diagnosis of HIV infection should never be made on the basis of ELISA positivity alone. Reactive ELISA tests require specific confirmation with a Western blot or other equally specific test, such as the *immunofluorescent assay* (IFA) for antibodies. The Western blot test measures antigen-antibody reactions to electrophoretically separated proteins of HIV. When antibodies to typical protein bands (e.g., p24, p31, and gp41 or gp160) are all present, the test is considered positive. A positive test generally means that a patient is infected with HIV and is infectious to others via sexual or blood-borne routes of transmission. When fewer bands are present, interpretation is difficult, and repeat testing and clinical correlation are required.

Recent reports have led to concern about the reliability of the ELISA and Western blot tests. Because they measure patients' antibodies to HIV, these tests are negative during an early "window period" between initial infection and antibody production.[3,4] This period has been estimated at 6 weeks to 3 months in most cases, with some patients remaining antibody negative for 6 months or longer. Imagawa, et al.[5] recently studied a select group of homosexual men who remained seronegative despite continuing to engage in high-risk sexual activity. Using viral cultures, 23 percent of the men in this particular group were infected with HIV. Retrospective polymerase chain reaction testing of stored sera from some of these men showed the presence of HIV infection up to 35 months before the time the positive viral culture was obtained.

During the first few months of the "window period," the *HIV p24 antigen* may be present in the serum as a marker of infection. This test may provide indirect evidence of infection shortly after at-risk activities or accidental exposure to blood or bodily fluids. Repeat antibody testing in subsequent months is required to confirm the diagnosis. Many blood banks use p24 antigen tests in addition to standard antibody tests to help detect units of blood obtained from persons in this early "window period."

The *polymerase chain reaction* (PCR) is a process in which viral DNA sequences are repeatedly doubled until there are enough copies to be detected. Peripheral blood mononuclear cells are

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used. Because of the amplification process, the test can be positive even when fewer than one cell in 10,000 is infected. However, laboratory cross-contamination of specimens has caused occasional false-positive results. False-negative results appear to be rare. The PCR is especially helpful in identifying actual HIV infection in infants, in whom positive antibody tests may reflect either HIV infection or passive transfer of maternal antibodies.

HIV viral cultures are difficult to perform, with 10–20 percent false-negative results. Along with the PCR test, cultures are most helpful in establishing the presence of HIV infection in the infant.

Comment

The combination of ELISA plus Western blot or IFA testing remains the standard diagnostic method for establishing the presence of HIV infection in adults. When properly performed, these tests appear to have sufficient sensitivity and specificity to be considered quite accurate. However, a "window period" exists before the development of antibodies. Although most persons infected with HIV will become seropositive by 3 months of infection and 95 percent will become seropositive by 6 months, a few may remain antibody negative for prolonged periods of time.[3-5] Therefore, caution must be exercised in patient counselling. Regardless of the results of testing, safe sex guidelines and avoidance of intravenous drug use must be emphasized.

Laboratory Indicators of Disease Progression

The clinical course of HIV disease, like most chronic diseases, varies considerably from patient to patient. Some conditions, such as oral candidiasis, lymphadenopathy, and even Kaposi's sarcoma (when it is the initial manifestation of HIV infection) may exist for years before the disease progresses. Other conditions, such as cytomegalovirus retinitis and toxoplasma encephalitis, tend to occur late in the course of the disease. A number of tests have been used to help estimate the rate of disease progression and the stage of disease. Following some of these laboratory findings may be helpful in patient management. Tests include CD4 (T-helper) lymphocyte counts, p24 antigen levels, and beta$_2$-microglobulin levels.

CD4 (T-helper) lymphocyte counts are the most widely used measurement for following the progression of HIV disease. CD4 lymphocytes are selectively destroyed by HIV infection, and their loss is key to the development of immunodeficiency. Normal CD4 cell counts vary enormously from person to person and among different laboratories. In a person infected with HIV, CD4 cell counts drop significantly at the time of HIV seroconversion. This decline is followed by a plateau lasting months or years. At the end of this plateau phase, cell counts drop by about 85 CD4 cells/μL per year.[6] In the San Francisco cohort study, only 16 percent of patients with more than 400 CD4 cells/μL progressed to AIDS within 3 years, whereas 46 percent with 201–400 cells/μL and 87 percent with fewer than 200 cells/μL developed AIDS within the 3-year follow-up period.[6] Most patients with CDC-defined AIDS have fewer than 200 CD4 cells/μL, and critically ill patients generally have fewer than 100 cells/μL.

Beta$_2$-microglobulin is a protein released when cell membranes rupture. Serum levels increase as disease progresses, including during the early stages of progression from asymptomatic to symptomatic disease.

HIV p24 antigen is generally undetectable in asymptomatic HIV-infected persons and usually remains undetectable until significant symptomatic disease occurs. Marked rises in p24 antigen levels may predict progression to AIDS within months. Clinical correlation is required, as this antigen may also be detected in about 6 percent of persons with other viral illnesses, such as cytomegalovirus infections.

Comment

There are no laboratory tests that are reliable enough to serve as "surrogate markers" to predict progression of HIV disease in individual patients. However, patients and physicians often wish to follow some laboratory values to help in clinical management. Most widely used are CD4 lymphocyte counts. A single measurement of CD4 cell counts may be very inaccurate, so repeat measurements to follow trends are essential. CD4 studies every 6–12 months for asymptomatic patients with counts greater than 500 cells/μL and every 3–6 months for symptomatic patients and those with fewer than 500 cells/μL seem to be reasonable guidelines. Other tests may be helpful

in selected cases but are not routinely required for primary care of the HIV-infected patient. Beta$_2$-microglobulin levels generally correlate with advancing disease, but patient management decisions should not be based on this value alone. HIV p24 antigen levels remain low or undetectable throughout most of the prolonged asymptomatic phase of HIV infection, and rapidly rising levels usually do not occur until obvious clinical progression of HIV is apparent. Both the beta$_2$-microglobulin and the p24 antigen tests have been used to monitor the effectiveness of antiviral therapy against HIV, although more data are required to assess the usefulness of these tests in practice.

HIV disease is a chronic disease. The time between infection and the development of clinical AIDS probably averages 8 to 10 years. Laboratory tests of disease progression can provide helpful but not precise information. Patients and providers need to understand that the interpretation of these tests must be made in conjunction with an overall clinical assessment and used only as one component of a comprehensive patient care plan.

Surrogate Markers and New Recommendations for Zidovudine in Asymptomatic HIV Infection

In August 1989, the director of the National Institute of Allergy and Infectious Diseases, Anthony S. Fauci, M.D., announced that studies of zidovudine (AZT) showed the drug to be effective in asymptomatic HIV-infected persons with CD4 counts between 200–500 cells/μL. For these asymptomatic patients, 500 mg daily appeared to be equally effective and less toxic than higher dosages. The details of the study, however, were not released at the time of the announcement, so many questions remain. In October, the study was submitted for publication (P. Volberding, personal communication) and data should be available for critical review shortly.

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