EDUCATIONAL COMMENTARY – TESTING FOR INFECTIOUS MONONUCLEOSIS

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Learning Outcomes

Upon completion of this exercise, the participant will be able to:

- Describe the symptoms of infectious mononucleosis.
- List the assays used to diagnose infectious mononucleosis.
- Discuss the sensitivity and specificity of assays used to confirm the diagnosis of infectious mononucleosis.
- Interpret the results of heterophile or specific Epstein-Barr virus antibody assays.

Infectious mononucleosis (IM) is an acute, self-limiting disease in which the Epstein-Barr virus (EBV) infects B lymphocytes, the WBCs responsible for the production of antibodies. Common symptoms include sore throat, fever, malaise, swollen lymph nodes, enlarged spleen, and inflammation of the liver. In rare cases EBV infection may cause severe complications, such as thrombocytopenia, hemolytic anemia, pericarditis, myocarditis, or neurological syndromes. Accurate diagnosis is important because these symptoms are present in several other infectious diseases, such as cytomegalovirus, adenoviruses, toxoplasmosis, and streptococcal infections. Unlike IM, some of these infections can be easily and effectively treated.

Most infections occur in early childhood and are asymptomatic. Infections in adolescents and young adults comprise the majority of symptomatic infections. Symptoms usually resolve without treatment within 2 months, but the virus becomes latent and persists for life. The virus may reactivate later but usually recurs without symptoms.

Diagnosis of infectious mononucleosis is based on clinical symptoms and the results of hematological and serological testing. WBC counts are usually elevated, and the lymphocyte count is increased. Large numbers of variant or reactive lymphocytes are present on the peripheral blood smear. These lymphocytes are not abnormal but their appearance changed when they reacted to the viruses.

Serodiagnosis of EBV infection is accomplished by the demonstration of heterophile antibodies or specific antibodies directed against components of the EBV. The fastest and most cost-effective methods for confirming a diagnosis of infectious mononucleosis are heterophile antibody assays. Heterophile antibodies are antibodies that will cross-react with antigens found on the erythrocytes of cattle, sheep, and horses, but will not react with guinea pig kidney cells. These antibodies are primarily IgM and appear in the serum of the patient about 1 week after the onset of infection. Antibody levels peak 2 to 4 weeks
after infection and decline to undetectable levels within 12 weeks. Heterophile antibodies may be detected using slide agglutination assays with either RBC or latex particles as antigen carriers. Solid-phase immunoassays may also be used to detect heterophile antibodies.

Testing for heterophile antibodies has been available for more than 50 years. The RBC carrier assays require that the patient serum be absorbed by beef erythrocytes and horse or guinea pig kidney cells prior to the addition of indicator horse erythrocytes. Heterophile antibodies found in serum from patients with IM are absorbed by beef erythrocytes but are not absorbed by guinea pig or horse kidney cells. Therefore, when the indicator horse erythrocytes are added, little or no agglutination will occur in the sample with the antibodies absorbed by the beef erythrocytes, but agglutination will occur in the sample with the antibodies previously absorbed by the guinea pig kidney cells. False-positive results occur in patients with rubella, malaria, viral hepatitis, systemic lupus erythematosus, and leukemia. Most latex agglutination assays employ a latex bead coated with purified bovine RBC extract as the antigen. The patient sample is added to the latex-coated bead. The test is positive if agglutination occurs. A single heterophile antibody titer does not indicate the severity of the disease, but serial determinations for a patient may be used to follow the course of the disease.

Approximately 85% to 90% of adult patients with IM produce heterophile antibodies. The sensitivity of heterophile antibody assays is very low when testing children <4 years of age because, in most cases, they are unable to produce heterophile antibodies. Also, heterophile antibodies are demonstrable in only 25% to 50% of children up to age 12. Heterophile antibody assays have high specificity but lack sensitivity in the early phases of the infection. False-negative results occur in up to 25% of cases if testing occurs within the first week after infection. When testing 2 weeks after infection, the false-negative rate is about 5% to 10% and falls to 5% after 4 weeks.

Indirect immunofluorescence assay for EBV-specific antibodies is considered the "gold standard" technique for diagnosing IM. The procedure detects antibodies directed against specific antigens of EBV. Antibodies include anti-EBV early antigen (EA), IgM anti-viral capsid antigen (VCA), IgG anti-VCA, and anti–Epstein Barr nuclear antigen (EBNA). Presence of anti-EA and IgM anti-VCA indicate a current infection. Presence of IgG anti-VCA and anti-EBNA indicate a past infection. This procedure is labor intensive, slow, and expensive. Therefore, routine IM testing is performed using heterophile testing or solid-phase immunoassays specifically known as enzyme immunoassays (EIA) or enzyme-linked immunosorbent assays (ELISA). In addition to indirect immunofluorescent techniques, antibodies directed against specific EBV antigens may be detected using EIA or ELISA techniques.

Several studies have compared heterophile latex or erythrocyte agglutination tests for heterophile antibodies and solid-phase immunoassays to the reference method, indirect immunofluorescence.
has been determined that tests using purified antigens provide higher sensitivity than heterophile assays in diagnosing primary IM. Most solid-phase immunoassays and latex agglutination assays use purified bovine RBC extracts and, therefore, have greater sensitivity.

Heterophile antibody assays are cost-effective, reliable methods for confirming the diagnosis of IM. Testing for antibodies to specific EBV antigens is performed on patients who test negative for heterophile antibodies but who have clinical symptoms that indicate IM. These patients include children and immunocompromised patients, who are unable to produce heterophile antibodies.

References


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