EDUCATIONAL COMMENTARY – DIAGNOSING LYME DISEASE

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

- Discuss the history of Lyme disease.
- Name the causative agent.
- Name the vector of the disease.
- State the symptoms of the disease.
- Describe screening and confirmatory testing for Lyme disease.
- Discuss treatment.

It is commonly thought that Lyme disease was discovered in the late 1970’s in Lyme, Connecticut. Actually, similar cases were described as far back as 1883 in Germany. In 1976 in Lyme, Connecticut, a large number of children were misdiagnosed with juvenile rheumatoid arthritis. The persistence of a group of mothers led public health officials to further investigate this disease cluster. In 1977, the disease was named Lyme arthritis. Since most cases were diagnosed in the summer, it was speculated that the disease was transmitted by an arthropod. A researcher, Willy Burgdorfer, at the United States Rocky Mountain Laboratories of the National Institutes of Health, found spirochetes (spiral-shaped bacteria) in the body fluid of *Ixodes* ticks. Dr. Burgdorfer and Dr. Alan Barbour successfully cultured the spirochete in 1984. The causative agent of Lyme disease was then named *Borrelia burgdorferi*.

In nature, *B. burgdorferi* is found in white-footed mice. The *Ixodes* tick bites the mouse and then bites other animals, such as humans, deer, dogs, or horses. The primary host for these ticks is the white-tailed deer. Most cases in humans occur after the bite of an infected deer tick. The disease can be transmitted by any stage of the tick, but the nymph stage is responsible for most cases. This stage is most active in the summer. The tick is about the size of a pin head and must be attached for at least 24 hours to transmit the disease. Ninety-five percent of cases of Lyme disease occur in the northeastern, mid-Atlantic, and north central states. There are over 23,000 cases reported each year. Individuals who work or live near wooded areas with high populations of deer are at risk of contracting Lyme disease.

Lyme disease begins with a localized red, slowly expanding, ring-like rash. The rash appears from 2 days to 2 weeks after the tick bite and fades within 3-4 weeks even if left untreated. Then flu-like symptoms occur including fever, headache, and fatigue. Later, the infection may cause inflammation of the heart, central nervous system disease, such as meningitis or facial palsy, or arthritis. Symptoms vary widely from patient to patient making diagnosis difficult.
Diagnosis of Lyme disease is made after evaluation of the patient’s history and clinical symptoms, followed by laboratory testing for the infection. Determining an accurate diagnosis is hampered by the following: (1) about 20% of patients do not exhibit the rash, (2) it is difficult to culture the bacteria from skin scrapings or body fluids, and (3) antibody is not detectable in the patient’s serum soon after infection.

The first antibody produced against *Borrelia burgdorferi* is IgM. This class of antibody peaks approximately 3-4 weeks after infection. Testing prior to 7 days after infection detects only about 30% of the cases. IgG antibodies are not detectable until 4-6 weeks after the appearance of the rash. Initial testing for Lyme disease should be performed by enzyme-immunoassay (EIA) or immunofluorescent assay (IFA). In the immunofluorescent assay, the slide is coated with the spirochete and the patient’s serum is added. The slide is washed to remove all serum that is not bound to the spirochete antigen. Then anti-human globulin with a fluorescent tag is added and the slide is washed again. The slide is viewed with a fluorescent microscope. A positive specimen will produce fluorescence with a titer of 256 or greater. This procedure is limited by the occurrence of false negatives and false positives. Testing performed on patient specimens too soon after infection may produce false-negative results. False positive results may occur in several other conditions including syphilis and rheumatoid arthritis. The test is also time-consuming and requires a high degree of expertise.

More commonly, enzyme immunoassay is used to screen patients for Lyme disease. This method is rapid, sensitive, and relatively inexpensive. Antigens from the bacteria are coated on micro-titer wells in a strip. Patient serum is added to the well and incubated allowing the patient’s antibodies to attach to the *B. burgdorferi* antigens. The well is washed to remove all antibodies in the patient’s serum except those attached to the antigen. An antibody-conjugate consisting of anti-human globulin with an enzyme tag is added. An enzyme substrate is then added and the well is incubated again. A colored reaction is produced which is read on a spectrophotometer. The more color produced, the greater the concentration of antibody in the patient’s serum. This method may also produce false negative results on specimens collected early in the infection. False positive results may occur in individuals with syphilis, infectious mononucleosis, Rocky Mountain spotted fever, and some autoimmune diseases.

In 1999, a rapid test for Lyme disease that may be performed in physicians’ offices became available. This test, called PreVue, is an immunochromatographic technique that screens for IgG and IgM antibodies to recombinant antigens of *B. burgdorferi*. Patient serum or whole blood is added to the membrane, and then a diluent is added. The serum and the diluent dissolve the antibody-binding proteins found on the membrane and all IgG and IgM antibodies in the patient serum become labeled with color. The labeled antibodies migrate to the antigen band in the test window that contains recombinant antigens to *B. burgdorferi*. If the patient has specific antibodies to *B. burgdorferi*, a colored line will appear in the test window. The color-labeled antibodies continue to migrate across the membrane to the control window. A colored line will form in this window regardless of the presence of specific antibodies.
to *B. burgdorferi*. A positive patient result requires a colored line in the test window and a colored line in the control window. If a colored line only appears in the control window, the patient sample is negative.

Specimens which give negative results using the approved screening methods, EIA or IFA, require no further testing and should be reported as negative for Lyme disease. Positive or questionable results from screening procedures should be followed by testing using the standardized Western Blot procedure. In this technique, *Borrelia* antigens are electrophoresed on acrylamide gel to separate the proteins according to their molecular weight. The antigens are then transferred onto nitrocellulose paper and the patient’s serum is allowed to react with the antigens on the nitrocellulose strip. If the patient serum has an antibody to any of the separated antigens, the antibody will bind to that antigen. An enzyme-labeled anti-human globulin is overlayed on the strip. This globulin will bind to any patient antibody bound to the strip. An enzyme substrate is then added. Color will form a band at the location of all bound patient serum antibodies. The patient’s strip is compared to a standard strip. A specified number of bands must be present to report the specimen as positive.

Polymerase chain reaction (PCR) testing may also be performed but is only performed in reference laboratories. This procedure allows for the identification of *Borrelia* DNA from a skin sample excised from the area of the rash. PCR is expensive, labor intensive, requires considerable expertise to perform, and has low sensitivity when testing blood, spinal fluid, or other specimens. In the future, this testing may become more commonly used.

Antibiotic therapy is used to treat individuals diagnosed with Lyme disease. Effective antibiotics include penicillin, cephalosporin, and tetracycline. A single dose of antibiotic may prevent Lyme disease if administered to the patient soon after the tick bite.

*Reference*


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