Microbiological diagnosis of Lyme borreliosis

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SUMMARY

Lyme borreliosis is a complex multisystem disorder. Clinical manifestations are variable and differential diagnosis is often difficult. Besides clinical criteria (erythema migrans), the diagnosis of Lyme borreliosis can be ascertained by demonstration of borrelial infection using either direct (isolation, PCR) or indirect (serology) microbiological tests. Isolation of *B. burgdorferi* sensu lato from clinical material represents the most reliable method for confirming borrelial infection. PCR has been developed for the detection of *B. burgdorferi* sensu lato DNA in clinical specimens. In contrast to culture, PCR is a rapid method but it is not standardized. Serologic tests represent the most commonly used method for establishing microbiological diagnosis of borrelial infection. Specific IgM and IgG antibodies can be detected in blood, CSF and synovial fluid. The percentage of seropositivity increases with duration of infection. It is not possible with serologic tests to distinguish between acute, late, active, or treated disease. In patients with Lyme borreliosis a specific T-cell response to *B. burgdorferi* sensu lato can also be detected.

KEY WORDS

Lyme borreliosis, diagnosis, isolation, PCR, serology

Introduction

Lyme borreliosis is a complex multisystem disorder that affects persons of all ages and both sexes. Infection can manifest with protean clinical signs; different organs can be affected, including the skin, nervous system, joints, and others (1). Clinical manifestations are variable and differential diagnosis is often difficult. The best clinical marker of the disease is the initial skin lesion erythema migrans (1, 2). In some patients clinical manifestations are not specific but indicate a borrelial etiology, in others they can be entirely non-specific for borrelial infection. In these cases, microbiological confirmation of borrelial infection is essential (3).

Besides clinical criteria (primarily the presence of erythema migrans), the diagnosis of Lyme borreliosis can be ascertained by demonstration of borrelial infection using either direct (isolation, PCR) or indirect (serology) microbiological tests. Each individual method has its own sensitivity and specificity; criteria for the evaluation are based on clinical parameters. In many cases different tests are combined.

Cultivation

Borrelia burgdorferi sensu lato has been isolated from different clinical specimens: skin, CSF, blood, synovial fluid etc. during early as well as chronic stages of Lyme borreliosis (4). Specimens for isolation must be taken under aseptic conditions and before the institution of antimicrobial therapy. In clinical specimens, *Borreliae* are present in low numbers and/or periodically (for example in blood), thus specimens for isolation have to be as large as possible (like 2 ml of CSF, 10 ml of blood etc.). Because *Borreliae* are susceptible to the environmental conditions it is recommended to inoculate specimens into the medium as soon as possible (e.g. bed side) (3, 4).

Borreliae grow optimally in modified Kelly medium at 33°C; several variants of basic Kelly's medium have been developed (5-7). Because borrelial generation time is long and ranges from 8 to 24 h (it depends primarily on borrelial adaptation to the artificial medium) it is recommended to cultivate specimens for at least nine weeks (2,4-7). For many physicians cultivation is not very attractive because it is a demanding, long lasting, and expensive procedure. However, isolation of *B. burgdorferi* sensu lato from clinical material represents the most reliable method for confirming borrelial infection, especially in patients with unspecific clinical manifestations.

Although *B. burgdorferi* sensu lato grows well in laboratory conditions, it is not easily recovered from clinical specimens. The frequency of recovery of *Borreliae* from skin biopsies ranges from up to 40% in patients with acrodermatitis chronica atrophicans to 70% in patients with erythema migrans (4). Biopsies taken from the expanding edge of erythema migrans yield comparable culture positivity as biopsies taken from the centre of the skin lesion (8). The frequency of recovery of *Borrelia* from other specimens (CSF, blood, synovial fluid etc.) is less then 10%. Overgrowth of competing bacteria (for example bacteria of normal skin flora) may be a serious problem in culturing *Borrelia* from clinical specimens. Additionally, contamination of samples during long cultivation process also decreases the frequency of recovery (4).

Although *Borreliae* are fastidious and requesting for cultivation, this method showed some findings important for elucidating pathogenesis of borrelial infection:

I.) *Borreliae* have been isolated from blood and CSF of patients with solitary (and multiple) erythema migrans. These findings confirm that *Borreliae* disseminate early after infection (9-11).

II.) *Borreliae* have been isolated from patients with high antibody titers such as patients with acrodermatitis chronica atrophicans (12). It seems that specific antibodies as demonstrated by serological tests are not able to eradicate *Borreliae* from the body. Moreover, in these patients *Borreliae* were isolated not only from skin but also from CSF, indicating multiorgan affection.

III.) *Borreliae* have been isolated from patients that had been treated with antimicrobial agents appropriately (13). Although *Borreliae* are susceptible to antibiotics, they have some mechanisms to survive treatment.

IV.) Genotypic and phenotypic characterisation of isolated strains show different distribution of species regarding the geographical regions (Europe, USA) as well as biological material (humans, ticks), and indicate rough association of borrelial species and clinical manifestation of Lyme borreliosis (*B. afzelii* with skin disorders, *B. garinii* with neurologic involvement) (10,14-16).

Polymerase chain reaction (PCR)

PCR has been developed for the detection of *B. burgdorferi* sensu lato DNA directly in body fluids and tissue specimens (17). The target sequence for amplification can be borrelial chromosomal DNA (16S rRNA gene, flagellin gene etc.) or plasmid DNA (e.g. gene for OspC, OspA etc.) (17-19). For detection with PCR, it is not necessary that borrelial strains are alive; it is sufficient that their DNA is preserved. Thus, it is reasonable to perform PCR even in patients treated with antibiotics.

In contrast to culture, PCR is a rapid method that can give results in hours. But, like the culture, PCR results also depend on the concentration of spirochetes in the specimen taken for analysis. Although successful detection of less than 10 microorganisms in the sample has been reported, in some patients culture seems to be more sensitive than PCR (20-21). Inhibitory substances in the specimens and sample preparation procedures may negatively influence PCR sensitivity (22).

Specificity of PCR is determined mainly by the choice of specific primers and probes. Borrelial DNA heterogeneity and significant sequence differences in the target gene can cause false-negative results (23). On the other hand, false-positive results are possible mainly because of extremely high sensitivity of the procedure: they can be a result of airborne contamination with borrelial DNA (primarily already amplified DNA) (24).

PCR may be helpful for the diagnosis of Lyme borreliosis but it remains a non-standardised method. PCR protocols differ regarding to the sample preparation, target DNA selection, primer selection, selection of amplifying method, and detection of PCR-generated products. Thus, PCR results should be interpreted with caution and according to clinical findings and the efficiency of the test.

Serology

At present, serologic tests represent the most commonly used method for establishing microbiological diagnosis of borrelial infection. Methods include enzyme-linked immunosorbent assay (ELISA), immunofluorescent assay (IFA) and Western immunoblot (WB). Specific IgM and IgG antibodies can be detected in blood, CSF and synovial fluid.

Humoral antibody response to *B. burgdorferi* sensu lato is complex and varies individually. Specific IgM antibodies can be detected after 3 to 6 weeks, IgG antibodies some weeks later. Low sensitivity of tests at the beginning of the infection is a consequence of late onset of antibody production. The percentage of seropositivity increases with the duration of infection. About one fourth of patients with early infection (like erythema migrans) and almost all with chronic infection (such as acrodermatitis chronica atrophicans) are seropositive (3,25). In general, some patients develop a strong, the others a weak immune response, while in some patients an immune response can not be detected. On the other hand, in some healthy persons, especially from endemic regions, specific antibodies can also be detected. Absence of immune response does not mean the absence of borrelial infection and, on the contrary, presence of an immune response does not indicate active infection (3,4). Serologic tests also do not distinguish between acute, late, active, or treated disease.

Phenotypic heterogeneity of *Borrelia* strains has pronounced impact on the antibody formation and detection. An infected person produces antibodies directed against the antigens exhibited by the infecting strain. Because of different antigen profiles and considerably distinct antibody responses, serologic tests must be capable to detect quite heterogeneous antibody responses evoked by different borrelial strains (26). Commercial as well as home-made serologic tests are not standardised. These tests use different *Borrelia* species (*B. afzelii, B. garinii*, and *B. burgdorferi* sensu stricto) or different strains within the same species as test antigen. Serologic tests also vary regarding the antigen preparation: whole *Borrelia* strain, purified, sonicated, or recombinant borrelial antigens can be used (16,27).

False positive reactions occur particularly in patients with syphilis or relapsing fever, and in patients with autoimmune diseases. Possible cross-reaction with antigens from a broad range of microorganisms can influence test results. Some of the cross-reactive antibodies can be reduced by adsorption tests (4,25).

Cell-mediated immunity

Investigations of cell-mediated immunity in patients with Lyme borreliosis showed specific T-cell responses to *B. burgdorferi* sensu lato. Some patients demonstrate a significant cell-mediated immune response while they are only borderline or low seropositive to *Borreliae.* Although applicability of T-cell proliferative assay is controversial, it may be a diagnostically useful in some groups of patients with Lyme borreliosis (28-29).

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