DETECTION OF BORRELIA BURGDORFERI BY POLYMERASE CHAIN REACTION IN THE BIOLOGICAL FLUIDS

E. Ružić-Sabljić

SUMMARY

Low bacterial loads and difficult growth in culture, slow antibody response, persistence of specific antibody response even after asymptomatic infections and negative results of serological tests are characteristic for *B. burgdorferi* infection. To facilitate the understanding of *B. burgdorferi* infection the PCR has been applied as a method capable of elucidating the pathogenesis of infection and as additional method of confirming borrelial infection. The PCR is a method for amplifying specific nucleic acid sequences by use of repeated cycles of DNA synthesis.

Under appropriate conditions, the PCR can be used to identify *B. burgdorferi* in human tissues and biological fluids; less than 10 microorganisms in samples can be identified. The specificity is determined by the choise of an optimal DNA sequence of *B. burgdorferi* as a target sequence for the amplification. Applicability of the PCR for routine diagnostic procedure should be established by more detailed investigations.

KEY WORDS

Polymerase chain reaction, B. burgdorferi, blood, cerebrospinal fluid, urine

Lyme borreliosis (LB) is characterized by a wide range of clinical manifestations caused by a spirochetes of the genus *Borrelia: B burgdorferi, B. garinii* and *B. afzelii* (1,2).

In a borrelial infection, a relatively low concentration of spirochetes in clinical samples is characteristic and may result in an unsuccessful isolation procedure (3). *B. burgdorferi* can be cultivated from the skin in about 50 % of the cases, the recovery rate from cerebrospinal fluid (CSF) is 7-10 %, and only 3 % from blood. Because cultivation is a low-yield, time consuming and expensive procedure, it is not the method of choice to confirm the infection. In clinical practice serologic tests are usually used (4, 5). However, because of a slow antibody response early LB can be missed, and on the other hand, because of persistence of specific antibody response which may be longstanding even after asymptomatic

infections, some other diseases may be falsely interpreted to be of borrelial origin. Negative results of serological tests do not rule out the presence of the causative agent nor do specific antibodies guarantee the presence of *B. burgdorferi* in the host (6). To facilitate the understanding of the role of *B. burgdorferi* in different stages of LB, the polymerase chain reaction (PCR) has been applied as a method capable of elucidating the pathogenesis of infection and as an additional method of confirming infection (7).

The polymerase chain reaction (PCR) is a powerful technique which may detect nucleic acids of specific microorganism. It can detect about 10-100 bacteriae in a clinical sample, and with a nested PCR the sensitivity increases (8, 9). This "in vitro" technique is used to amplify specific DNA sequences of interest, the general sequence of the gene has to be known.

The basic PCR cycle is as follows (7):

1. Denaturation. Double stranded DNA is separated into single strands by heating to 95°C.
2. Annealing. Two oligonucleotide primers (about 20 nucleotides in length) are required each flanking the opposite end and the opposite strand of the DNA template. The optimal temperature required for annealing ranges between 45°C and 70°C.
3. Polymerase extension. Excess quantities of the four deoxynucleotide triphosphates (dATP, dTTP, dGTP and dCTP) and enzyme Taq polymerase are added to the mixture. Once the primers have annealed to the complementary DNA sequence, the polymerase will extend them from the 5' towards the 3' end. New DNA is synthesized complementary to the corresponding DNA template.

The cycle of denaturation, annealing and chain extension is repeated through approximately 25 - 40 cycles. The number of copies of amplified DNA increases exponentially with each cycle, leading to as many as 10^9 copies after 30 cycles.

Low bacterial loads and difficult growth in culture are characteristic of *B. burgdorferi* infection. Successful detection of *B. burgdorferi* by the PCR is reported in samples with less than 10 microorganisms (7). This assay is capable of amplifying directly lysed *B. burgdorferi* without purifying the DNA; hence, extensive sample preparation is not required. Sensitivity of the PCR is also retained in the presence of human DNA, thus it should be applicable to the detection of *B. burgdorferi* in human specimens (10).

The specificity of the PCR is determined by the choice of an optimal DNA sequence of *B. burgdorferi* as a target sequence for the amplification and by the careful choice of a pair of primers to initiate synthesis on each strand of a DNA template. Amplification produces millions of copies of the target DNA sequence which can be visualized, cloned or sequenced. The most frequently used target DNA sequences of *B. burgdorferi* are genes encoding 16S rRNA (11), flagellin (9,12,13), outer surface protein A (OspA) (7,8) and OspB (7), but any other DNA sequence can also be used (10). The DNA sequence chosen for amplification may influence the specificity of the reaction. It is more relevant to amplify those DNA regions which are specific to the pathogen on the species level, such as OspA, OspB or OspC genes. By amplifying genus specific DNA regions, such as flagellin or 16S rRNA genes, it is possible to identify other *Borreliae* beside those associated with LB (11,13). Molecular biology reports a high degree of homology as well as the lack of sequence conservation among the genes, so not only the choice of the gene, but also the choice of the region of the gene for amplification can influence on the specificity of the PCR (9,13). Results of the PCR always have to be contemplated in accordance with the clinical signs present in a patient. Under appropriate conditions, the PCR can be used to identify *B. burgdorferi* in human tissues and biological fluids.

1. PCR detection in blood.

B. burgdorferi can be disseminated from a primary skin lesion early in the course of illness, even in the first few days (14). Dissemination is rarely manifested with clinical signs or symptoms, probably because of a low number of *Borreliae* in the blood (1). This also results in an unsuccessful isolation procedure (3). The PCR successfully identified *B. burgdorferi* in blood and confirmed suspicion of the early dissemination of the pathogen in some patients with EM (15). If a distinction between early borrelial skin lesions (EM, unspecific lesions, borrelial lymphocytoma) with and without dissemination of the causative agens was possible, it would be much easier to advise on the appropriate antibiotic treatment. There is no need for PCR detection in blood in cases of EM; it can be more useful in cases of unspecific manifestations suspected to be of borrelial origin (16,17). A greater part of these patients have negative serological tests, as well as negative cultivation procedure and doubts may arise how to treat them properly. It is expected that in some cases the PCR can identify borrelial DNA in blood at times of dissemination; it could probably be more effective when used directly in tissue.

In patients with chronic LB, the PCR can demonstrate the presence or the absence of the spirochaetemia which would help in the elucidation of the pathogenesis of chronic borrelial infections or it may be used for monitoring the effectiveness of the antibiotic therapy.

One of the properties of the *B. burgdorferi* is the adherence to a number of eucaryotic cell types (18), so, on the PCR detection in blood may influence the adherence of *B. burgdorferi* to the blood cells and platelets. The real impact of this adherence on the efficiency of the PCR should be investigated in detail, in any cases, it must be considered.

2. PCR detection in the cerebrospinal fluid

Neurological involvement of LB may affect both the peripheral and central nervous systems (CNS), causing a wide range of acute or chronic neurological manifestations. Some clinical phenomena are typical for LB, such as Bannwarth's syndrome, others are non-specific and borrelial infection has to be confirmed by the demonstration of a specific immune response or by the isolation of *B. burgdorferi* (1,3,4). The PCR in the CSF can elucidate the role of this bacteria in the pathogenesis of neuroborreliosis, confirm borrelial infection and point out interactions between *B. burgdorferi* and its host (19).

Using the PCR, it was shown that *B. burgdorferi* can disseminate into the CNS early in the course of LB, at the time when EM is present, with minimal or no clinical evidence of CNS involvement. At the time of dissemination, many of the patients still have no specific immune response and the CSF chemistry and cell counts in the CSF may be normal (20,21). Confirmation of the presence of borrelial DNA in the CSF as well as in the blood in some cases of eariy LB gives great value to the PCR. The PCR results enable the selection of an appropriate antibiotic therapy.

The PCR may be of value in some cases of early neuroborreliosis, like in the case of acute lymphocytic meningitis, which is sometimes the only manifestation of LB. To determinate the etiology of meningitis after a tick bite can be a real problem for clinicians, especially in areas such as Slovenia which are endemic for several tick-borne diseases with neurological involvement (22, 23). Beside *B. burgdorferi*, meningitis can be caused by other pathogens transmitted by ticks, for example by Arboviruses. In patients with meningitis due to a tick bite, specific antibodies against *B. burgdorferi* in the serum and the CSF may not be present in the first days or weeks of the illness, and isolation of *B. burgdorferi* from the CSF is low-yield procedure. With the PCR, it could be possible to detect DNA of *B. burgdorferi* in the CSF and to confirm the borrelial origin of the meningitis. It would be very interesting to compare the results of isolation, serology and PCR detection of *B. burgdorferi* and other etiological agens in CSF in patients with lymphocytic meningitis in a prospective study.

In cases of chronic neuroborreliosis, such as borrelial disseminate encephalomyelitis, diagnosis could usually be established by the detection of specific antibodies in the CSF and the serum (4, 5). In these patients, the PCR is not necessary for diagnosing infection, it can be used to study the pathogenesis of chronic neuroborreliosis and eventually for monitoring the effectiveness of antibiotics (12, 19). In chronic neuroborreliosis it is not necessary that all the CSF specimens are positive by the PCR. Unsuccessful PCR detection indicate the absence of *B. burgdorferi* in the CSF and may be the consequence of *B. burgdorferi* adherence to the cells of glial origin as well as the result of locally synthesized antibodies (24).

3.PCR detection in synovial fluid

Joint involvement in LB may occur early after infection, frequently as arthralgia, rarely as migratory arthritis. Early joint involvement is often accompanied by other more specific clinical borrelial manifestations. The knee seems to be most commonly involved, followed by other large joints. Lyme arthritis is characterized by brief recurrent attacks of asymmetric swelling and pain (1). As a rule specific borrelial antibodies are elevated (4, 5). As in other biological specimens, isolation of *B. burgdorferi* from synovial fluid is unsatisfactory and is not the method of choice for diagnosing borrelial infection (3, 25). Unsuccessful isolations from synovial fluids can be explained by the possible persistence of *B. burgdorferi* in the synovial membrane rather then in synovial fluids, and only sometimes can *Borreliae* be found in the synovial fluid. It is supposed that the PCR can detect such a low number of bacteria in synovial fluid, but for the moment there are not many reports (7, 26). The use of the PCR in synovial fluid can be compared with its use in cases of neuroborreliosis: it could be used for studying the pathogenesis of infection and eventually for monitoring the effectiveness of antibiotic therapy.

CONCLUSIONS

1. *B. burgdorferi* can be successfully detected in biological fluids by the PCR. On the laboratory level, this technique could be maximally optimized in its sensitivity and specificity by choosing an appropriate DNA sequence of *B. burgdorferi* for amplification, by using an appropriate set of primers and by improving optimal reaction conditions.

2. The PCR can confirm early dissemination of *B. burgdorferi* from the primary lesion, suggesting the necessity of early antibiotic treatment.

3. Beside serological tests and the cultivation procedure, the PCR in organic fluids can be used for confirming borrelial infection.

4. The PCR could be especially valuable in cases where borrelial infection can not be confirmed by serological tests and the cultivation technique

5. The applicability of the PCR can be widened to study the pathogenesis of the infection and eventually for monitoring the effectiveness of antibiotics.

6. The real value and applicability of the PCR for routine diagnostic procedure should be established by more detailed investigations.

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AUTHOR'S ADDRESS

Eva Ružić Sabljić, MD, MSc, Institut of Microbiology
Medical Faculty, University of Ljubljana, Zaloška 4, 61000 Ljubljana, Slovenia