

# Bone Marrow as a Source for *Borrelia burgdorferi* DNA

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## ABSTRACT

The diagnosis of persistent Lyme disease has depended on the detection of serum antibody to *Borrelia burgdorferi*. Patients may lose their immune response over time or it may be abrogated by antimicrobial therapy. These case reports

describe patients with chronic Lyme disease and a reactive bone marrow polymerase chain reaction (PCR). After appropriate and aggressive treatment, specific DNA may persist in sequestered sites such as bone marrow.

**Key words:** polymerase chain reaction, outer surface protein A gene, *B. burgdorferi* DNA

## INTRODUCTION

Diagnosis of Lyme disease by laboratory methods continues to be difficult. Most tests rely on the production of IgM and/or IgG antibody to *Borrelia burgdorferi*. Antibody production may be abrogated by antimicrobial agents<sup>1</sup> or may not occur at all. IgM and IgG antibody responses may fluctuate over time. Complicating these immune responses is the lack of reliability of many of the commercially available serological tests.<sup>2,3</sup> Only recently<sup>4</sup> has standardization of confirmatory Western blot testing been discussed in an organized forum and recommendations published for the use of proficiency panels.<sup>5</sup>

There is a need for methods to directly detect *B. burgdorferi* in body fluids. Gene amplification, notably polymerase chain reaction (PCR), satisfies the requirements for both sensitivity and specificity. Several reports document the advantages of PCR for detection of DNA in synovial fluid<sup>6</sup> cerebrospinal fluid<sup>7,8</sup> as well as other body fluids such as blood and urine.<sup>9</sup> Relatively few, if any reports, have cited bone marrow as a diagnostic specimen.

The following case reports describe patients who had a reactive PCR for *B. burgdorferi* DNA in the bone marrow and positive *B. burgdorferi* antibody tests.

## Case #1

This 51-year-old female was seen for the first time in April 1989 with a Lupus-like illness. There were insufficient clinical and laboratory data to fulfill the American Rheumatology Association criteria for Systemic Lupus Erythematosus (SLE). She was treated with nonsteroidal anti-inflammatory agents. From October 1989 to January 1991, she reported frequent respiratory infections, sinusitis, increasing joint pains, headaches, and fatigue. She was started on Plaquenil (hydroxychloroquine sulfate) for autoimmune arthritis in March 1991, but was symptomatic through June of 1991 with frequent upper respiratory infections on intermittent antimicrobial agents. By September 1991, she complained of severe headaches, fatigue, nausea, joint swelling, and urinary symptoms. She was again placed on various regimens of oral antimicrobials in addition to Plaquenil and tapering doses of steroids.

In June 1992 she was admitted to the hospital with chest pain (left hilar) and aorticopulmonary adenopathy. All of her laboratory tests were within normal limits, except antinuclear antibodies (ANA), which were present at a titer of 1:40. The gallium scan was positive in the left hilum and mediastinum. An abdominal CT scan was unremarkable. A biopsy of a mediastinal lymph node revealed noncaseating granulomas, which were atypical for sarcoidosis. Progressive improvement was seen through January of 1993 when she developed an acute influenza-like illness associated with severe arthritis and neck stiffness. The patient was started on oral Biaxin (clarithromycin) and Suprax (cefixime) for presumptive

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seronegative Lyme disease. Initiation of treatment was followed by a flare of an acute neurologic syndrome including vertigo, headaches, and profound fatigue. She became progressively worse until March 1993 when she began intravenous antimicrobial (IV) therapy with Claforan (cefotaxime). By that time her ANA was repeatedly negative but she experienced multiple Herxheimer reactions on Claforan requiring adjustment of dosage.

After 2 months of IV antimicrobial agents, her condition improved. She had no additional antimicrobials until March 1994 when she developed gradually recurring symptoms of headaches, joint pains, fevers, paresthesia, profound fatigue, and difficulty with word-finding. The ANA's remained either negative or weakly positive. An ELISA antibody test for Lyme disease was performed with a commercial kit and was reactive on June 2, 1994 but was not confirmed by Western blotting. She was maintained on Zithromax (azithromycin) and Plaquenil. She continued to have severe fatigue, requiring total bed rest. Immunological studies were normal except for IgG and lambda monoclonal gammopathy. In September 1994, a bone marrow biopsy was performed. The PCR for *B. burgdorferi* DNA was reactive. She was again started on IM 1.2 mu BiCillin (penicillin) on October 3, 1994 and oral Zithromax 250 mg BID. The patient was maintained on the above regimen until clinical remission with progressive improvement of all her symptoms. Repeat immunological studies continue to be within normal limits.

#### Case #2

This 59-year-old female was seen for the first time on April 8, 1994 after being treated by another physician for Lyme disease. She had multiple influenza-like illnesses in 1992 through 1993 and was treated with short courses of oral antibiotics. She went to Spain in 1993. This trip was followed by the onset of severe pain and weakness. The patient had been a professional singer and could no longer sing. When tested for Lyme disease, the IgM Western blot was reactive. She was started on treatment for Lyme disease in August 1993 consisting of IV Claforan (Cefotaxime) for 8 weeks. She initially felt better but then relapsed. Intravenous ampicillin was administered for 1 month followed by Claforan (9 g/d) for 2 weeks which was reduced to 6 g/d. By May of 1994, there was lack of response and she was started on IV vancomycin. The patient responded well but relapsed when vancomycin was discontinued. Vancomycin was restarted in June of 1994 and the patient responded well.

Therapy was continued through July followed by pulse vancomycin and BiCillin LA (penicillin). By August 1994, she was very symptomatic with fevers and arthritis. The IV vancomycin was stopped and Plaquenil started in

addition to intramuscular (IM) BiCillin. The patient was noted to have hypogammaglobulinemia and anemia and treated with IV gammaglobulin and transfusions. The patient did well until October 1994 when both symptoms and anemia recurred. The consulting hematologist performed a bone marrow biopsy that was unremarkable except for a positive PCR (*B. burgdorferi*). By November 1994 she had recurrent fevers and arthritis. Her anemia is stable on Epogen (epoetin alpha) and her strength is improving on Plaquenil. She has had two gammaglobulin transfusions for recurrent hypogammaglobulinemia. Throughout this time, both her IgG and IgM Western blots have remained consistently positive. The patient now reports that she is feeling better.

#### DISCUSSION

*B. burgdorferi* DNA was detected from bone marrow samples of these patients. The DNA was amplified with specific primers for the highly conserved OspA (outer surface protein A) gene sequence of *B. burgdorferi*. The amplification and detection system used detected 10 copies of the OspA gene in the sample. The unique OspA primers are highly specific for *B. burgdorferi* and do not cross-react with closely related organisms such as *B. hermsii*. Known positive serum and urine samples, as well as a standard curve of *B. burgdorferi* DNA are included in each assay to confirm assay sensitivity and reproducibility throughout the sample processing, amplification and detection steps. Since PCR can be adversely affected by inhibitors of Taq polymerase commonly found in clinical specimens, all samples are required to pass a strict inhibition test that consists of the amplification, and detection of a small amount of a known DNA sequence added to an aliquot of the test sample. Samples that fail the inhibition test are repurified and retested. Negative controls included a series of buffer controls, extracts from normal human cells, and negative patient material. All negative controls must test negative before the PCR process can be validated. Amplification products were detected using a proprietary microtiter-based capture hybridization assay for the OspA gene. This system generates a strong signal with greater sensitivity than conventional hybridization formats. Southern blot hybridization with nonradioactive alkaline phosphatase probes specific for the OspA sequence, was used as a confirmatory method for amplicon detection.

To assure the validity of the sensitive PCR system, stringent quality control practices were observed. Sample processing, preparation, and reagent formulation functions were carried out in separate areas within the laboratory dedicated exclusively for PCR. Reagents were pretested and prealiquoted for one time usage. Further precautions included the use of uracil-N-glycosylase to minimize amplicon cross-contamination. In cases such as

these, the presence of *B. burgdorferi* DNA can be detected in patients with active infection. Body fluids have been shown to contain small levels of *Borrelia*, which may be difficult to detect by culture, but may be detectable with the use of the PCR assay that is capable of detecting as little as the equivalent of less than 10 organisms per mL of fluid.<sup>9</sup> This laboratory performs more than 2500 PCR tests per month for a variety of infectious agents and has never had a contamination incident. Manak et al<sup>10</sup> suggested that PCR reactivity could be cyclical. That is, *B. burgdorferi* DNA may be released into the circulation in small amounts at unpredictable time intervals but apparently associated with antibiotic therapy. They also reported that reactive PCR tests may be observed in patients with no detectable antibody to *B. burgdorferi* and who fulfilled the Centers for Disease Control's criteria for Lyme disease. Of 16 patients with chronic Lyme disease and a reactive PCR, only 4 were seropositive.

The significance of *B. burgdorferi* DNA in the bone marrow is unclear. Some investigators believe that *B. burgdorferi* is an intracellular bacterium. It is possible that the bone marrow serves as a reservoir for the Lyme disease agent and that sporadic appearance of nucleic acid in the circulation occurs after a DNA shower from the bone marrow, possibly stimulated by antimicrobial induced cell lysis. Thus, the bone marrow biopsy, albeit invasive, may prove to be a clinically significant procedure in a patient whose signs and symptoms are not sufficiently focused to rule out other diseases.

Unlike many suspected Lyme disease patients, these 2 individuals had a serologic diagnosis of Lyme disease and received extensive and seemingly adequate antibiotic treatment over a several-year period. They developed hematologic abnormalities, which resulted in a bone marrow biopsy. Symptoms of Lyme disease persisted in spite of aggressive treatment.

This report demonstrates several phenomena:

1. Despite aggressive IV and oral antimicrobial treatment, *B. burgdorferi* may persist in sequestered areas of the body such as bone marrow.

2. The yield on PCR testing for Lyme disease may be enhanced by testing bone marrow specimens or, by

extrapolation, testing leukocytes, ie, buffy coat, where the organism may reside intracellularly protected from immune defenses. The bone marrow provides a specimen resource rich in nucleated cells, which may harbor the spirochete in dormant or active form. We clearly do not advocate this invasive testing for all patients but rather those who are refractory to standard therapy and have concomitant immunologic or hematologic abnormalities.

3. There does not appear to be a clear correlation between reactive Lyme serology and PCR positivity, although one patient had a persistently positive IgG and IgM Western blot and the other a positive ELISA test.

In conclusion, these case reports suggest that bone marrow may be an important site for detection of *B. burgdorferi* DNA.

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