

The Spectrum of Gastrointestinal Manifestations in Children and Adolescents with Lyme Disease

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ABSTRACT

A clinical diagnosis of Lyme disease was made in 15 consecutive patients between the ages of 8 and 20 years who presented with a history of an erythema migrans rash followed by chronic gastrointestinal symptoms and multiple organ system complaints. Endoscopic evaluation was performed to assess the gastrointestinal mucosa and to obtain biopsies for polymerase chain reaction (PCR) to the outer surface protein A (Osp A) of *Borrelia burgdorferi*. As age matched controls, 10 patients with biopsy-proven Crohn's disease were also tested by PCR. The laboratories assessing the histopathology and performing the PCR were blinded to the diagnosis of all specimens.

The presence of *B burgdorferi* DNA in the gastrointestinal tract was confirmed by PCR in all of the patients with the clinical diagnosis of Lyme disease who had chronic gastrointestinal symptoms and in two control subjects with Crohn's disease. Biopsy evidence of chronic gastritis, chronic duodenitis, and chronic colitis was found in patients with Lyme disease who had chronic gastrointestinal symptoms and was associated with the presence of *B burgdorferi*.

The chronic gastrointestinal symptoms that occurred within 6 months of an erythema migrans rash and Lyme disease may be attributed to a direct effect or immune mediated response to *B burgdorferi*.

Key words: Lyme disease, abdominal pain, blood in stool, *Borrelia burgdorferi*, gastritis, duodenitis, colitis, polymerase chain reaction

INTRODUCTION

Lyme disease affects a wide range of organ systems, producing dermatologic, musculoskeletal, neurologic, genitourinary, lymphatic, hepatic, renal, respiratory, cardiovascular, and ocular manifestations.^{1,2} One report to date describes the presence of *Borrelia burgdorferi* in the stomach, intestines, and colon of children.³ To further address the clinical manifestations of Lyme disease and the possibility of direct involvement of the gastrointestinal (GI) tract, a prospective study was made of 15 consecutive patients who had a physician documented ery-

thema migrans (EM) rash followed by chronic gastrointestinal symptoms and multiple organ system complaints of Lyme disease.

METHODS

All patients included in our study had a physician documented EM rash with no prior history of gastrointestinal complaints. They were referred to the pediatric gastroenterology and nutrition service of Jersey Shore Medical Center for evaluation of chronic abdominal pain, chronic diarrhea, acid reflux, or blood in the stool that occurred within 6 months after the onset of the EM rash. From January 1998 through April 1999, 15 consecutive patients satisfying the above clinical criteria⁴ were evaluated prospectively. There were 6 boys and 9 girls evaluated (mean age 14 ± 3.6 years, range 8-20). Each case included a history, physical examination, complete blood cell count, liver function tests, sedimentation rate, antinuclear antigen (ANA), HLA B27, esophagogastroduodenoscopy (EGD), and/or colonoscopy. A Lyme Western blot was performed

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for confirmation of an acute (immunoglobulin M) or past (immunoglobulin G) *B burgdorferi* infection. A positive IgM Western blot was interpreted as 2 of 3 bands (23, 39, 41 kd). A positive IgG Western blot was interpreted as 5 or more of the following *B burgdorferi*-specific bands: 18, 23, 28, 31, 34, 39, 41, 45, 58, 66, 93 kd. A diet history was taken to assess the dietary fat intake. Ultrasonography of the abdomen was performed when the history suggested a diagnosis of gallstones or pancreatitis. Stool samples were examined for occult blood, *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, ova and parasites, and *Clostridium difficile* toxin. Gastrointestinal (GI) biopsies were reviewed to assess the mucosa by microscopy and whether *Helicobacter pylori* (on EGD only) or eosinophilia was present.

Biopsy specimens were taken from areas of the GI tract that looked inflamed during EGD or colonoscopy. The biopsies were assigned randomly to three histopathologists who were blinded to the diagnosis of the specimens they received. The histopathologists did not perform a silver stain for the detection of spirochetes because it is not routinely done. Biopsies were reported as acutely inflamed when polymorphonuclear cells were present in the mucosa and chronically inflamed if 6 or more plasma cells and lymphocytes were present in the gastric mucosa without polymorphonuclear cells. Chronic duodenitis or chronic colitis was diagnosed when more than 6 intraepithelial lymphocytes per 100 surface absorptive cells were present in tissue biopsies in conjunction with a distortion in glandular architecture.

Polymerase chain reaction (PCR) for DNA to *B burgdorferi*⁵ was performed on all biopsies by Medical Diagnostic Laboratories in Mount Laurel, New Jersey. In all patients in which *B burgdorferi* DNA was detected, PCR for *B burgdorferi* RNA polymerase was performed and results are reported in the Table. As a target for DNA amplification, the gene coding for the outer surface protein A (OspA) of *B burgdorferi* was selected and analyzed as described below.

DNA Isolation from Biopsy Specimens

Total DNA was extracted from duodenal, gastric, and colonic biopsies as described by Maniatis et al.⁶ The samples were centrifuged (5 minutes, 4°C, 14K rpm) and the pelleted biopsy was subjected to 500 µL of cell lysis buffer [0.5% SDS, 470 µL TE buffer, 5 µL of proteinase K (20 µg/µL)]. The samples were incubated for 24 hours at 50°C. Proteinase K (5 µL) was added to the mixture every 6 hours. DNA was extracted by phenol chloroform, followed by ethanol precipitation. DNA concentrations were determined spectrophotometrically by measuring the A_{260} .

DNA Amplification

The SL primers (SLA 5'-AAT AGG TCT AAT AAT AGC CTT AAT AGC-3' SLB 5' CTA GTG TTT TGC

CAT CTT CTT TGA AAA-3') are suitable for amplification of all *B burgdorferi* sensu lato isolates. The SL primers amplify a region (nucleotide 21-328) of the *B burgdorferi* sensu stricto B31 OspA sequence. One µg of isolated DNA was used as a template DNA in the presence of a 20 pmol sample of each primer in a 50 µL reaction mixture. The samples were subjected to 35 amplification cycles in a Perkin Elmer 2400 thermocycler (Foster City, CA) under the following conditions: 93°C, 1 minute; 65°C, 1 minute; and 72°C, 1 minute. PCR amplification products were resolved onto 1.5% agarose electrophoresis gels and visualized under ultraviolet light with ethidium bromide.

To test the presence of inhibitory substances and to provide a positive control in the PCR assay, amplifications were also performed with primers targeting the histone gene. A positive control was performed with every biopsy specimen. It included a PCR in the presence of 100% *B burgdorferi* DNA that was purchased from the American Type Culture Collection (Rockville, MD). This *B burgdorferi* DNA was isolated from *Ixodes scapularis* tick, New York Type strain, and shipped frozen to the laboratory.⁷ The negative control performed with each biopsy specimen included the PCR in the absence of DNA. A second genomic DNA control is done weekly at the laboratory as part of their quality control. Physical containment measures ensured the absence of DNA contamination in the PCR procedure.

As age-matched controls, 10 adolescents with biopsy proven Crohn's disease (5 boys, 5 girls, 13.5±2.5 years, range 10-17), who had not been on antibiotics one year prior to endoscopy, were also tested by PCR. The laboratory performing the PCR analysis was blinded to the diagnosis of all specimens they received.

Statistical Analysis

The sensitivity and specificity of PCR for the detection of *B burgdorferi* in the GI tract was calculated. The confidence intervals (CI) were calculated by using the Fischer's Exact test method. A Fischer's exact test was used to determine the association between inflammation and PCR positivity in each of the biopsied sites.

RESULTS

Patients with Lyme disease presented with chronic abdominal pain (n=10, 67%), chronic diarrhea (n=1, 7%), visibly evident blood in the stool (n=2, 13%), and acid reflux with heartburn (n=2, 13%). In all 4 patients whose biopsies revealed evidence of colitis, the abdominal pain was characterized as a crampy, perumbilical pain that started at the right middle quadrant of the abdomen and spread to the left middle quadrant of the abdomen or vice versa. The pain was unrelated to meals and occurred

Table. The gastrointestinal manifestations and biopsy results of patients with Lyme (1-15) and Crohn's disease (A-J).

Patient	Antibiotics (months)*	Chief complaint	Gastric biopsy	Duodenal biopsy	Colon biopsy	Gastric PCR [†]	Duodenal PCR	Colon PCR
1	5	abdominal pain	gastritis	duodenitis	colitis	DNA	DNA	DNA/RNA
2	4	blood in stool	gastritis	(-)	colitis	DNA	(-)	DNA
3	4	acid reflux	(-) [‡]	(-)	NB	DNA	(-)	NB
4	3	abdominal pain	gastritis	(-)	(-)	DNA	DNA	(-)
5	5	abdominal pain	gastritis	duodenitis	(-)	(-)	DNA	(-)
6	5	abdominal pain	(-)	(-)	NB	DNA/RNA	(-)	NB
7	2	diarrhea	(-)	(-)	colitis	DNA	(-)	DNA
8	0	abdominal pain	gastritis	(-)	(-)	(-)	(-)	DNA/RNA
9	2	abdominal pain	gastritis	(-)	(-)	(-)	(-)	DNA/RNA
10	0	abdominal pain	gastritis	(-)	NB	DNA	(-)	NB
11	0	blood in stool	NB [§]	NB	colitis	NB	NB	DNA/RNA
12	1	abdominal pain	gastritis	duodenitis	NB	(-)	DNA	NB
13	2	abdominal pain	(-)	(-)	NB	DNA	(-)	NB
14	0	abdominal pain	(-)	(-)	NB	(-)	DNA/RNA	NB
15	0	acid reflux	gastritis	(-)	NB	DNA	(-)	NB
A	0	blood in stool	(-)	(-)	granuloma	(-)	(-)	(-)
B	0	abdominal pain	(-)	(-)	granuloma	(-)	(-)	DNA
C	0	abdominal pain	gastritis	duodenitis	granuloma	(-)	(-)	(-)
D	0	abdominal pain	(-)	(-)	granuloma	(-)	(-)	(-)
E	0	abdominal pain	gastritis	duodenitis	granuloma	(-)	(-)	(-)
F	0	abdominal pain	granuloma	(-)	granuloma	(-)	(-)	(-)
G	0	blood in stool	NB	NB	granuloma	NB	NB	DNA
H	0	blood in stool	NB	NB	granuloma	NB	NB	(-)
I	0	abdominal pain	gastritis	duodenitis	granuloma	(-)	(-)	(-)
J	0	abdominal pain	(-)	(-)	granuloma	(-)	(-)	(-)

*Number of months of antibiotic treatment for Lyme disease prior to GI biopsy.

[†]PCR-DNA denotes the detection of *B. burgdorferi* DNA (the outer surface protein A) by PCR of biopsy specimens. DNA/RNA denotes the detection of *B. burgdorferi* DNA and RNA polymerase by PCR of biopsy specimens.

[‡](-) denotes no histological pathology detected on biopsy or no detection of *B. burgdorferi* by PCR.

[§]NB denotes an area of the gastrointestinal tract that was not biopsied and a PCR test that was not performed due to the absence of a specimen from that site.

Abbreviations: DNA = deoxyribonucleic acid; NB = not biopsied; PCR = polymerase chain reaction; RNA = ribonucleic acid.

throughout the day. In the remaining 6 patients with abdominal pain whose biopsies revealed gastritis, duodenitis, or both, the abdominal pain was characterized as periumbilical, burning, and improved by avoiding fried foods and foods high in fat content. Ultrasonography of the abdomen did not reveal any gallstones or evidence of pancreatitis. In 2 patients who complained of acid reflux, their pain was a burning midepigastic pain that radiated to the esophagus. The pain occurred within the first post-prandial hour and was relieved by antacids. Ten of the 15

patients with Lyme disease had evidence of inflammation at a biopsy site with detection of *B. burgdorferi* DNA at that site. Patients 2 and 11 had blood in their stool and presented with the clinical features of Crohn's disease (ie, 15 pound weight loss in a year, arthritis of the knee, protein losing enteropathy) and ulcerative colitis (6 bloody bowel movements a day for a week), respectively. The biopsies of all the patients with Lyme disease revealed no evidence of granulomas or terminal ileitis. In patient 6, the IgM Western blot was positive and showed

the 23, 31, 34, 39, 41, 58, and 66 kd bands. The IgG Western blot was negative (no bands present). No other patient had a positive Western blot. All control patients with Crohn's disease had biopsy proven terminal ileitis and granulomatous colitis. Lyme disease was diagnosed in 15 patients and 2 with Crohn's disease had a positive PCR to *B burgdorferi* DNA in biopsy specimens from the gastrointestinal tract (Table). In 6 patients with Lyme disease, *B burgdorferi* DNA was detected in the GI tract and *B burgdorferi* RNA polymerase was detected by PCR.

A positive *B burgdorferi* PCR occurred with chronic inflammation in the GI tract of 11 of 15 patients with Lyme disease. In patients 8 and 9, inflammation occurred in the stomach; however, *B burgdorferi* DNA was detected in the colon. *B burgdorferi* DNA was detected in the GI tract in the absence of inflammation in 4 patients (27%), 3 of whom had received at least 2 months of antibiotics prior to endoscopy. There was no statistically significant association between PCR positivity in the GI tract and chronic inflammation.

In 10 of 15 patients (67%), antibiotic therapy for Lyme disease had been prescribed within 1 to 5 months prior to endoscopy (n=4, 1-2 months; n=3, 3-4 months, and n=3, 5 months). Despite prior antibiotic use, all 4 patients with colitis were PCR positive for *B burgdorferi* DNA in the colon while 5 of 9 with gastric inflammation were PCR positive in gastric biopsies. *Helicobacter pylori* was not detected in any of the gastric biopsies. *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, and *Clostridium difficile* toxin was not detected in any of the stool samples. HLA B27 was positive in patients 1 and 11 and in none of the controls. ANA was positive and had a speckled pattern in patients 2, 5, and 6. An elevated sedimentation rate of 85 and 28 were found in patients 4 and 13, respectively.

The lab performing the PCR had a false positive rate of 1 in 500 by analyzing 6550 specimens from January 1998 through April 1999. The sensitivity of GI *B burgdorferi* DNA detection was 100% (15/15) with a 95% CI (81.9%, 100%). The specificity was 80% (8/10) with a 95% CI (44.4%, 97.5%). The positive predictive value was 88.2% (15/17) with a 95% CI (63.6%, 98.5%).

DISCUSSION

Abdominal pain and the associated GI pathology in children with Lyme disease whose biopsies are PCR positive for *B burgdorferi* has not been reported previously. The presence of an EM rash in the past or a positive Western blot and chronic GI symptoms in the past does not mean that the two are related. However, positive detection of the OspA gene in biopsies confirmed the presence of *B burgdorferi* DNA in the biopsied tissue samples while offering the advantage of no cross reaction

with other spirochete species that have been previously detected in the GI tract.⁸⁻¹⁰ It is possible that PCR, a highly sensitive method, could lead to false positive results because of the amplification of similar sequences of related microorganisms. However, a false positive rate of 1 in 500 biopsy specimens suggests that this occurs infrequently.

B burgdorferi may contribute to GI symptoms by its presence directly in the GI tract or by eliciting an inflammatory or immune response.^{11,12} In 11 patients (73%), inflammation in the GI tract was accompanied by a positive PCR to *B burgdorferi* suggesting an association between the infection and inflammation in these patients. In 2 patients the detection of the DNA occurred at a site distant from the inflammation. In the absence of inflammation, the presence of *B burgdorferi* may have contributed to abdominal pain and acid reflux (patients 3, 6, and 13).

Most available evidence suggests that appropriate antimicrobial treatment is highly efficacious to cure Lyme disease. As previously reported,^{13,14} we found that *B burgdorferi* persisted even after 1 to 5 months of antibiotic therapy. Despite prior antibiotic therapy, we were still able to detect *B burgdorferi* DNA and RNA polymerase in these patients. The detection of RNA polymerase in 5 patients suggests that the infection was actively replicating. In two cases (patients 1 and 6), this active replication occurred despite 5 months of antibiotic therapy for Lyme disease. Previous work has demonstrated that *B burgdorferi* can invade human fibroblasts and be protected from antimicrobial action.¹⁵ The ability of the organism to survive in this intracellular environment is one mechanism by which it may evade the immune response of the host and thus persist. Antibiotic resistance is another method that could explain the persistence of the organism despite prior antibiotic therapy. While the detection of *B burgdorferi* may represent evidence of prior or ongoing Lyme disease, it may not be the only etiology of the patients' abdominal symptoms.

Duray and Steere¹⁶ reported that *B burgdorferi* elicits interleukin-1, collagenase, prostaglandin E2, and circulating immune complexes. Some of these immune complexes may exert their effect at a distant site from the infection. Two patients (8 and 9) illustrate this possibility. In each case, *B burgdorferi* was detected in the colon but the inflammation was found in the stomach.

An inflammatory or immune reaction as a result of Lyme disease could affect the colon and may persist because of circulating immune complexes.¹⁶ Inflammatory and immune etiologies have also been proposed in Crohn's disease^{17,18}; however, this is the first report to describe the detection of *B burgdorferi* in patients with Crohn's disease. The role of *B burgdorferi* in patients with Crohn's disease needs further investigation.

CONCLUSION

Children and adolescents with a history of Lyme disease and chronic GI symptoms occurring within 6 months of an EM rash, had evidence of inflammation in the stomach, duodenum, and colon. We found *B. burgdorferi* by PCR of GI biopsies to be associated with chronic inflammation. The inflammatory reaction we describe may have been caused by spirochetes or by immune system products elicited in response to the spirochete presence.

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