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Misdiagnosis of Late-Onset Lyme Arthritis by Inappropriate Use of *Borrelia burgdorferi* Immunoblot Testing with Synovial Fluid

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The primary objective of this study was to determine whether patients with putative late-onset Lyme arthritis based upon synovial fluid *Borrelia burgdorferi* IgM and IgG immunoblot testing offered by commercial laboratories satisfied conventional criteria for the diagnosis of Lyme arthritis. Secondary objectives included assessing the prior duration and responsiveness of associated antibiotic therapy. We conducted a retrospective analysis of 11 patients referred to an academic medical center infectious disease clinic during the years 2007 to 2009 with a diagnosis of Lyme disease based upon previously obtained synovial fluid *B. burgdorferi* immunoblot testing. Ten of the 11 (91%) patients with a diagnosis of late-onset Lyme arthritis based upon interpretation of synovial fluid *B. burgdorferi* immunoblot testing were seronegative and did not satisfy published criteria for the diagnosis of late-onset Lyme arthritis. None of the 10 patients had a clinical response to previously received antibiotics despite an average course of 72 days. Diagnosis of Lyme arthritis should not be based on synovial fluid *B. burgdorferi* immunoblot testing. This unvalidated test does not appear useful for the diagnosis of Lyme disease, and this study reinforces the longstanding recommendation to use *B. burgdorferi* immunoblot testing only on serum samples and not other body fluids. Erroneous interpretations of "positive" synovial fluid immunoblots may lead to inappropriate antibiotic courses and delays in diagnosis of other joint diseases.

Lyme disease is a multisystem infection that in North America is caused by the tick-borne bacterial pathogen *Borrelia burgdorferi* (15). Although arthralgia and myalgia frequently accompany early Lyme disease, late-onset Lyme arthritis typically arises months after infection acquisition. Late-onset Lyme arthritis affects large, weight-bearing joints, with knee involvement nearly universal at some point, although other articulations may be involved. Current criteria for the diagnosis of late-onset Lyme arthritis are based on the presence of a characteristic clinical picture, exposure in an area where the disease is endemic, and positive serology indicating the presence of antibodies in the serum against *B. burgdorferi* (2, 12, 17). Serologic testing is particularly important, as 100% of patients with late-onset Lyme arthritis have strongly reactive two-tier testing with a positive total-antibody screen (enzyme immunoassay [EIA] or immunofluorescence assay [IFA]) and a positive IgG immunoblot (14). While a positive synovial fluid *B. burgdorferi* DNA PCR test provides adjunctive evidence implicating the pathogen, the test is not needed to secure a diagnosis, given its limited sensitivity (12).

An initial course of oral antibiotic therapy typically yields response rates of up to 90% for late-onset Lyme arthritis (13). Despite additional courses of antibiotics, a subset of patients develop persistent inflammation of the synovial joint without evidence of active infection that appears to be due to molecular-mimicry mechanisms.

Some commercial laboratories offer *B. burgdorferi* immunoblot testing using a serum-validated assay for other specimens, such as cerebrospinal fluid and synovial fluid, despite warnings against such use (3). Although anti-borrelial IgG antibodies have been described in synovial fluid of patients with Lyme arthritis, no published clinical data exist supporting the interpretation or clinical utility of synovial fluid *B. burgdorferi* immunoblots (5, 6). This study investigated 11 patients referred to a university-based clinic with persistent arthritis following a putative diagnosis of and treatment for Lyme disease based upon synovial fluid *B. burgdorferi* immunoblot testing. The patients were evaluated to determine whether they satisfied current clinical criteria for a diagnosis of Lyme arthritis.

MATERIALS AND METHODS

A retrospective review was performed of all patients referred to the infectious disease clinic of the Johns Hopkins University School of Medicine (JHUSOM) for evaluation of arthritis ascribed to Lyme disease based upon a synovial fluid *B. burgdorferi* immunoblot test obtained prior to the visit. Referring physicians who had performed the test included family practitioners, rheumatologists, and orthopedists. Persons who had not had a synovial fluid *B. burgdorferi* immunoblot test were excluded. Patients seen between 1 January 2007 and 31 July 2009 were eligible for inclusion. Study approval was obtained from the JHUSOM Institutional Review Board.

Demographic and relevant clinical data, including history and physical examination findings, were collected. The results of all laboratory tests and radiological studies were obtained retrospectively through retrieval of prior records or the usual clinical care. The study used criteria for the diagnosis of late-onset Lyme arthritis based upon the presence of a characteristic clinical picture of mono- or oligoarticular arthritis, including joint effusion, exposure in an area where the infection is endemic, and positive serum *B. burgdorferi* two-tier serological testing using EIA with an IgG immunoblot assay (12, 17). Serum testing ordered by study physicians was performed either in the laboratory of Johns Hopkins Hospital or in commercial laboratories, depending on individual patient insurer re-

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TABLE 1 Demographic and baseline historical information

Characteristic	Value
Age (yr) [mean ± SD (range)]	47.7 ± 11.9 (32–73)
Male [*n* (%)]	4 (36)
Caucasian [*n* (%)]	11 (100)
Preexisting conditions [*n* (%)]	6 (54)
Osteoarthritis	4 (36)
Rheumatoid arthritis	1 (9)
Spondyloarthropathy	1 (9)
Distribution of arthritis [*n* (%)]	
Monoarticular	6 (54)
Oligoarticular	5 (46)
Joint aspirated [*n* (%)]	
Knee	9 (82)
Wrist	1 (9)
Elbow	1 (9)
Course of arthritis/joint effusion [*n* (%)]	
Intermittent	11 (100)
Persistent	0 (0)
Time since onset of arthritis prior to consultation (days) [mean (range)]	1051 (122–2555)
History of erythema migrans rash [*n* (%)]	1 (9)
History of tick bite [*n* (%)]	1 (9)
Concurrent symptoms at time of consultation visit [*n* (%)]	
Arthralgia	11 (100)
Subjective cognitive dysfunction	6 (54)
Fatigue	4 (36)
Myalgia	2 (18)
Prescribed antibiotic courses[a]	
Total no. of days of antibiotic therapy for seronegative patients [mean (range)]	72 (10–165)
Seronegative patients treated with oral antibiotics[b] [*n* (%)]	10 (100)
Duration (days) [mean (range)]	53 (10–108)
Patients treated with parenteral antibiotics[c] [*n* (%)]	5 (45)
Duration (days) [mean (range)]	38 (2–70)
Patients simultaneously receiving intravenous and oral antibiotics [*n* (%)]	5 (45)

[a] Of the 10 seronegative patients.
[b] Doxycycline, amoxicillin, clarithromycin, or azithromycin.
[c] Ceftriaxone.

TABLE 2 Laboratory test results

Data on serum and synovial fluid samples	No. of patients
Serum	
Serological testing not performed before clinic presentation (*n*/11)	5
Seropositive by EIA and IgG immunoblot before presentation to clinic (*n*/6)	0
Seropositive by EIA and IgG immunoblot (*n*/11)	1
Seropositive by Lyme C6 peptide antibody (*n*/6)	1[b]
Synovial fluid[a]	
Positive *B. burgdorferi* DNA PCRs (*n*/2)	0
WBC count range (*n*/12)[c]	
≤200 cells/mm^3	2
201–2,000 cells/mm^3	4
2,000–6,000 cells/mm^3	2
>6,000 cells/mm^3	4

[a] Synovial fluid samples were analyzed by commercial laboratories using commercially available immunoblot kits for *B. burgdorferi* IgM and IgG.
[b] The same patient tested positive for EIA, IgG immunoblot, and Lyme C6 peptide antibody.
[c] One of the 11 patients had two synovial fluid samples tested from the same joint (separated in time). WBC, white blood cell.

quirements. Telephone calls were placed to commercial laboratories offering synovial fluid *B. burgdorferi* immunoblot testing to assess what studies, if any, had been performed to validate the test.

Patients who met the inclusion criteria for participation in the study were notified by postcard with a proviso to opt out of follow-up contact. Subsequently, all patients participated in providing self-reported clinical information. After agreeing to a brief telephone interview, note was taken of their arthritis activity and current diagnosis and whether additional courses of antimicrobial therapy had been prescribed since their initial evaluation by one of the JHU investigators.

The primary objective of this analysis was to determine whether the study patients met established diagnostic criteria for Lyme arthritis. Secondary objectives included a review of alternative diagnoses and clinical outcomes, as well as a tabulation of each patient's history of antibiotic use and subsequent response relevant to their reputed diagnosis of Lyme disease.

RESULTS

Eleven patients met the criteria for inclusion in our study. All of the patients were Caucasian adults, and most were women (7/11). All patients resided in the mid-Atlantic region of the United States and engaged in activities potentially exposing them to ticks in areas in which *B. burgdorferi* is known to be endemic. Synovial fluid samples had been taken from the knee in the majority (9/11), while two cases involved other joints (wrist and olecranon bursa). All patients gave a history of intermittent joint swelling and concurrent symptoms of some kind. The mean average duration of symptoms before initial consultation was 1,051 days (range, 122 to 2,555 days). Demographic and historical information is shown in Table 1.

Standard *B. burgdorferi* serology was performed in only 6/11 patients (55%) prior to presentation, none of which satisfied traditional two-tier EIA and immunoblot criteria for positive serum serology. All 11 patients had synovial fluid immunoblots that referring physicians had interpreted as indicative of active Lyme disease.

Following evaluation at JHU, only one patient, who had a history of erythema migrans but no prior serum testing, had a positive *B. burgdorferi* serum EIA and IgG immunoblot (Table 2). All patients had joint fluid cell counts assayed (Table 2). The median synovial fluid leukocyte count was 1,010 (range, 9 to 22,500) cells/mm^3. At least one band was detected on both the IgM and IgG immunoblots on each of the 12 analyses. At least two bands were detected in 11/12 (92%) IgM immunoblots, and at least five bands were detected on 4/12 (33%) of the IgG immunoblots (Table 3).

All patients had received antibiotic therapy previously. The single EIA and IgG immunoblot-seropositive patient was treated previously with doxycycline (120 days) and amoxicillin (30 days) with an ultimately curative response. The 10 seronegative patients were treated with antibiotics for an average of 72 days with a 0% antibiotic response rate at the time of evaluation by one of the study physicians (Table 1). In the seronegative group, the oral antibiotic most commonly employed was doxycycline (90%). One-half of these patients also received parenteral antibiotic therapy with ceftriaxone (duration range, 2 to 70 days). All seronega-

TABLE 3 Synovial fluid immunoblot results[a]

Immunoblot	No. of bands detected	Frequency [*n* (%)]	Band (kDa)	Frequency of specific band detection [*n* (%)]
IgM	0/3	0	23	10 (83)
	1/3	1 (8)	39	10 (83)
	2/3	6 (50)	41	8 (67)
	3/3	5 (42)		
IgG	0/10	0	18	4 (33)
	1/10	3 (25)	23	4 (33)
	2/10	2 (17)	28	5 (42)
	3/10	1 (8)	30	4 (33)
	4/10	2 (17)	39	3 (25)
	5/10	1 (8)	41	12 (100)
	6/10	0	45	6 (50)
	7/10	1 (8)	58	3 (25)
	8/10	1 (8)	66	5 (42)
	9/10	0	93	2 (17)
	10/10	1 (8)		

[a] Frequencies are based on *n*/12 patients. One of 11 patients had two synovial fluid immunoblots performed, and both are represented in the table. The one patient who was seropositive had 10/10 IgG bands, as well as 3/3 IgM bands, present in synovial fluid.

tive patients were recommended by the JHU investigators to cease antibiotic therapy.

The mean duration of study follow-up was 378 days (range, 19 to 885 days), with a median of 161 days. The seropositive patient reported complete resolution of arthritis without further antimicrobial treatment. Of the 10 seronegative patients, 8 (80%) self-reported the following etiologies for their arthritis since the time of their initial evaluation by the investigators: osteoarthritis (2), spondyloarthropathy (1), undifferentiated arthritis (1), tendon rupture (1), meniscal tear (1), bartonellosis (1), and "chronic Lyme disease" (1). Seven of the 10 seronegative patients reported that the severity of their arthritis had not changed since the time of their initial evaluation by the investigators. None of the total 11 patients reported continued use of antibiotics targeting Lyme disease, although two patients continued to believe their arthritis was due to Lyme disease or other suspected tick-borne infections.

A comprehensive Medline search (the terms used were Lyme disease, Lyme borreliosis, arthritis, *Borrelia burgdorferi*, synovial fluid, IgM, IgG, oligoclonal bands, Western blot, and immunoblot) found no study to date that had assessed the validity of the *B. burgdorferi* immunoblot assay on synovial fluid as a diagnostic test. When surveyed in August 2009, the two commercial laboratories that had performed the synovial fluid immunoblot testing confirmed that they had utilized FDA-approved immunoblot kits (intended for use with serum) on synovial fluid without any clinical validation studies.

DISCUSSION

This report not only serves as the first clinical description of *B. burgdorferi* synovial fluid immunoblots in the medical literature, but also highlights the potential pitfalls when using nonvalidated tests in an attempt to secure a diagnosis of Lyme disease. Of the 11 patients who presented with "Lyme arthritis" based upon synovial fluid *B. burgdorferi* immunoblot analysis, 10 (91%) were erroneously diagnosed. None of the 10 patients met the established criteria for a diagnosis of Lyme arthritis, as none were seropositive (compared to the usual 100% rate in late-onset Lyme arthritis) and most had fluid profiles not consistent with inflammatory arthritis typical of authentic *B. burgdorferi* infection (12). During follow-up telephone calls, two patients cited infectious explanations not accepted by mainstream medicine for their arthritis, as neither tick-borne *Bartonella* nor so-called "chronic Lyme disease" has been convincingly reported to cause authentic human disease (1, 7).

Besides delaying an accurate diagnosis, these initial mistaken Lyme disease diagnoses also led to lengthy courses of unnecessary antibiotic therapy that produced no clinical improvement, except in the one patient who had true infection based on a history of erythema migrans and confirmatory serotesting. One patient experienced an adverse drug reaction due to ceftriaxone. Inappropriate antibiotic therapy for suspected Lyme disease has been reported to cause bad outcomes (9, 10).

Despite laboratory report notation indicating that studies are for investigational purposes only, companies that offer synovial fluid immunoblotting merely add confusion regarding which test should be used to support a diagnosis of Lyme arthritis. No information is available as to how a diagnosis of Lyme disease was reached by the referring physicians; however, interpretive criteria were not offered on the laboratory reports. Of the six who had serum testing, all had negative results, so it appears that physicians read synovial immunoblots using conventional serum criteria (band patterns, ≥2/3 IgM or ≥5/10 IgG), misinterpreting or not obtaining serum testing. The physicians may also have disregarded the evidence-based Centers for Disease Control and Prevention recommendation not to use *B. burgdorferi* IgM immunoblot testing for patients with symptoms of more than 4 weeks duration due to high rates of false positivity, an error commonly made (4, 11). Why commercial laboratories list this nonstandardized test is unknown, but some offer such tests at a clinician's request. One commercial laboratory service manual did state that the synovial fluid immunoblot should be confirmed by conventional serum two-tier testing; however, this proviso was not listed on their Internet test menu or on the actual patient laboratory report (8). While the prevalence of synovial fluid *B. burgdorferi* immunoblot testing is not known, the Centers for Disease Control and Prevention have strongly cautioned against the use of unconventional, alternative, or nonvalidated tests for the diagnosis of Lyme disease (3).

Scant information exists regarding synovial fluid immunoblot band profiles in Lyme disease, and this information does not contain data that would support their use as a diagnostic aid (5, 6). Why banding patterns are seen in synovial fluid of seronegative patients is unknown, but it may be due to the sticky nature of the fluid in the assay (especially IgM immunoblotting) or is perhaps reflective of some immunopathogenesis by processes other than *B. burgdorferi* infection. Since the synovial compartment is not a protected site, there is an equilibrium with serum antibodies, as synovial fluid is mostly produced by plasma filtration (16). Moreover, absence of *B. burgdorferi* antibodies in the serum makes the diagnosis of Lyme arthritis unlikely, as others have found that antibodies are seldom produced only locally (6).

There are several limitations to this study. First, it is a retrospective case series and is based on a convenience sample of 11 patients. Second, all patients included in the study were suspected to have Lyme disease; a control group with synovial fluid for comparison (e.g., patients with inflammatory arthritides or healthy

controls) was not available. Third, the case series is subject to referral bias, especially in that nearly all patients had a non-antibiotic-responsive process. Despite these limitations, the high rate of inconsistency with existing diagnostic standards for Lyme arthritis suggests synovial fluid immunoblot tests are unlikely to prove clinically useful. Whether authentic intrasynovial production of *B. burgdorferi* antibodies may occur with negative serum studies is not known, but at least for the patients in this study, all such patients lacked a response to antibiotic therapy.

In summary, synovial fluid immunoblot testing should not be used for clinical evaluation of patients with suspected late-onset Lyme arthritis. While further study may more definitively address whether synovial fluid immunoblot testing has any diagnostic role, relying on such an unvalidated "positive" test result may well lead to misdiagnosis, unnecessary antibiotic courses, and delay in acquiring appropriate therapy. Finally, given the doubtful utility, commercial laboratories should withdraw *B. burgdorferi* synovial fluid immunoblots as an available clinical test. Laboratories should offer only testing that is validated and that follows published diagnostic recommendations.

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