Welcome to UVM ECHO: Lyme Disease and Tick-borne Illness

Facilitators: Mark Pasanen MD, Liz Cote
Faculty: Jean Dejace MD, Mark Levine MD
June 21, 2019
Introduction to ZOOM

• Mute microphone when not speaking
• Position webcam effectively
• Test both audio & video
• Communicate clearly during sessions:
  • Can use “raise hand” feature to comment
  • Speak clearly
  • Use chat function for technical issues
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No relevant disclosures

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<td>TeleECHO Session #2</td>
<td>• Early localized Lyme diagnosis, treatment, and interpreting tests</td>
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<td>TeleECHO Session #6</td>
<td>• Other tickborne diseases (babesiosis, etc.)</td>
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Goals for Session 2

1. Understand lab testing in Lyme Disease
   • Two-tiered testing

2. Diagnosing Early Localized Lyme Disease
   • Erythema Migrans
   • Diagnostic uncertainty

3. Treatment of early disease
Primer: Laboratory Testing In Lyme Disease

• Testing is imperfect
  • Lyme is difficult to culture
    • insensitive
    • takes several weeks to grow
  • Diagnosis is based on clinical presentation and serologic testing

• Sensitivity of serology (CDC 2-tiered testing)
  • Erythema Migrans: <50%
  • Early disseminated disease: ~80%
  • Late disease: >95%
Two-Tier Testing

• First: Enzyme Immunoassay
  • tests for IgG and IgM
  • rapid, easily automated
  • easy to interpret: positive/negative/equivocal
  • not as specific
    • cross-reacting Ab in e.g. syphilis, leptospirosis, mono, autoimmune disease, periodontal disease

• If positive or equivocal EIA, then Western Blot
  • Highly specific *when interpreted correctly
Two-Tier Testing

- Problems with the Western Blot
  - Costly
  - Subjective interpretation on the laboratory end
  - Most sent out to reference labs

- Results
  - 2 or more IgM bands = positive
  - 5 or more IgG bands = positive
High frequency of false positive IgM immunoblots for *Borrelia burgdorferi* in Clinical Practice

V. Seriburi, N. Ndukwe, Z. Chang, M. E. Cox and G. P. Wormser

Division of Infectious Diseases, New York Medical College, Valhalla, NY, USA

Abstract

Although it is known that two-tier serologic testing for Lyme disease may be associated with false positive results on the IgM immunoblot, this problem has never been systematically studied in the clinical practice setting. In a retrospective investigation of patients referred to the private adult practice of an Infectious Diseases physician for possible for Lyme disease, 50 of 182 patients (27.5%, 95% CI: 21.1–34.6) were found to have a false positive IgM immunoblot. 78.0% of these patients had received unnecessary antibiotic therapy. False positive results were not restricted to any single commercial laboratory. Research on alternative testing strategies that eliminate the IgM immunoblot entirely is warranted.
Two-Tier Testing

- Problems with the Western Blot
  - Costly
  - Subjective interpretation on the laboratory end
  - Most sent out to reference labs

Results
- 2 or more IgM bands = positive
- 5 or more IgG bands = positive

**FIG. 1.** Illustrative IgM immunoblot showing weak bands (The authors thank Dr. John Branda of Massachusetts General Hospital and Harvard Medical School, Boston, Mass. for providing this figure.) Immunoblot interpretation relies on two variables: band location and band intensity. Band location is judged by matching unknown bands against a band locator strip. Band intensity is measured by comparing unknown bands against a cutoff control band. In this figure, the band locator is labeled ‘BL’ and runs along the bottom. For an IgM immunoblot to be considered positive, reactivity with intensity equal to or greater than the cutoff control band must be present in at least two of the following three locations on the strip: 23 kDa, 39 kDa, or 41 kDa (arrows). Strip 1 was incubated with a positive control serum and displays sufficient reactivity at 23 and 41 kDa to be considered positive; there was only weak reactivity at 39 kDa. Strip 2 was incubated with the cutoff control serum and displays reactivity at 23 kDa only (arrowhead). Strip 3 was incubated with a negative control serum and has no visible bands. Strip 24 was incubated with a patient’s serum and displays several bands. However, all of the bands are of weaker intensity than the cutoff control band, and thus should not be scored as present. Furthermore, only one of the bands (23 kDa) is at an appropriate location to be relevant according to the CDC recommended IgM immunoblot criteria. Although there are weak bands in the general vicinity of 39 kDa and 41 kDa, they do not line up correctly.
FIG. 1. Illustrative IgM immunoblot showing weak bands (The authors thank Dr. John Branda of Massachusetts General Hospital and Harvard Medical School, Boston, Mass. for providing this figure.) Immunoblot interpretation relies on two variables: band location and band intensity. Band location is judged by matching unknown bands against a band locator strip. Band intensity is measured by comparing unknown bands against a cutoff control band. In this figure, the band locator is labeled 'BL' and runs along the bottom. For an IgM immunoblot to be considered positive, reactivity with intensity equal to or greater than the cutoff control band must be present in at least two of the following three locations on the strip: 23 kDa, 39 kDa, or 41 kDa (arrows). Strip 1 was incubated with a positive control serum and displays sufficient reactivity at 23 and 41 kDa to be considered positive; there was only weak reactivity at 39 kDa. Strip 2 was incubated with the cutoff control serum and displays reactivity at 23 kDa only (arrowhead). Strip 3 was incubated with a negative control serum and has no visible bands. Strip 24 was incubated with a patient’s serum and displays several bands. However, all of the bands are of weaker intensity than the cutoff control band, and thus should not be scored as present. Furthermore, only one of the bands (23 kDa) is at an appropriate location to be relevant according to the CDC recommended IgM immunoblot criteria. Although there are weak bands in the general vicinity of 39 kDa and 41 kDa, they do not line up correctly.
“Why do I have any bands?”
Serodiagnosis of Lyme Borreliosis by Western Immunoblot: Reactivity of Various Significant Antibodies against *Borrelia burgdorferi*

BINGNAN MA,* BEYAT CHRISTEN,† DANTON LEUNG,† AND CARMEN VIGO-PELFREY†

*Whittaker Bioproducts, Inc., Walkersville, Maryland 21793-0127*

Received 26 July 1991/Accepted 23 October 1991

The significance of various antibodies against *Borrelia burgdorferi* was studied by Western blot (immunoblot) by using 578 human serum samples. The proteins regularly detected by using samples from patients with Lyme borreliosis were those with bands with molecular masses of 94, 83, 75, 66, 60, 55, 46, 41, 39, 34, 31, 29, 22, and 17 kDa. The detectable frequencies of most of these proteins appeared to be significantly different between the sera from patients with Lyme borreliosis and those from normal control individuals as well as from the group with syphilis. The 39-kDa protein band recognized by polyvalent antibody was found to be the most significant marker for Lyme borreliosis. Furthermore, an anti-39-kDa immunoglobulin M response was detected in the samples from patients with early-stage Lyme borreliosis. Results from the use of monoclonal antibodies and patient sera revealed that the 39- and 41-kDa proteins may be structurally related but are immunologically distinct antigens. The significance of antibody reactivities to the 41-, 94-, 22-, 31-, and 34-kDa protein bands is also discussed.
FIG. 3. Comparison of the frequency of antibody reactivity to various *B. burgdorferi* protein bands between 186 patients with Lyme borreliosis and 320 normal controls.
Western Blotting in the Serodiagnosis of Lyme Disease

Frank Dressler,* Jennifer A. Whalen,* Bruce N. Reinhardt,* and Allen C. Steere

Division of Rheumatology/Immunology, Tufts University School of Medicine, New England Medical Center, Boston, Massachusetts

There are currently no accepted criteria for positive Western blots in Lyme disease. In a retrospective analysis of 225 case and control subjects, the best discriminatory ability of test criteria was obtained by requiring at least 2 of the 8 most common IgM bands in early disease (18, 21, 28, 37, 41, 45, 58, and 93 kDa) and by requiring at least 5 of the 10 most frequent IgG bands after the first weeks of infection (18, 21, 28, 30, 39, 41, 45, 58, 66, and 93 kDa). When these definitions were tested in a prospective study of all 237 patients seen in a diagnostic Lyme disease clinic during a 1-year period and in 74 patients with erythema migrans or summer flu-like illnesses, the IgM blot in early disease had a sensitivity of 32% and a specificity of 100%; the IgG blot after the first weeks of infection had a sensitivity of 83% and a specificity of 95%. Among patients with indeterminate IgG responses by ELISA, 6 of 9 patients with active Lyme disease had positive blots compared with 2 of 34 patients with other illnesses (P < .001). Thus, Western blotting can be used to increase the specificity of serologic testing in Lyme disease.
Western Blotting in 1

Frank Dressler,* Jennifer A. W
Bruce N. Reinhardt,* and Allen

There are current retrospective and
criteria was obtained
21, 28, 37, 41, 42
after the first week
definitions were
clinic during a 1
nesses, the IgM
blot after the first
patients with inc
had positive blot
blotting can be u

Figure 3. Receiver operating characteristic (ROC) curves for IgM and IgG Western blot criteria that gave largest ROC areas. Circles: sensitivity and 1 – specificity for 8 most common IgM bands in erythema migrans or Lyme meningitis. Triangles: same parameters for 10 most frequent IgG bands in Lyme meningitis, arthritis, or encephalopathy or polyneuropathy. Solid symbols show minimum number of bands needed for 99% specificity: 2 of 8 for IgM and 5 of 10 for IgG.

Lyme disease. In a
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in early disease (18,
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kDa). When these
ostic Lyme disease
ummer flu-like ill-
y of 100%; the IgG
ility of 95%. Among
fective Lyme disease
1). Thus, Western

Lyme Specialty Labs and Alternative Criteria

• As part of this investigation, blood from 40 healthy controls were sent to reference and specialty labs.
  • No history of prior diagnosis or treatment for Lyme
  • No history of Lyme-like symptoms
  • No history of another major medical disorder
  • Lack of residence or recent exposure to highly Lyme-endemic area

• 57.5% of healthy controls had a positive Lyme blot at one well-known specialty lab. None were positive by two-tier testing using CDC criteria.
A Comparison of Lyme Disease Serologic Test Results From 4 Laboratories in Patients With Persistent Symptoms After Antibiotic Treatment

Brian A. Fallon, Martina Pavlicova, Samantha W. Coffino, and Carl Brenner

Departments of Psychiatry, Biostatistics, Mailman School of Public Health, Neurology, Columbia University, and Lamont-Doherty Earth Observatory of Columbia University, Palisades, New York

(See the Editorial Commentary by Dattwyler and Arnaboldi on pages 1711–3.)

Background. As the incidence of Lyme disease (LD) has increased, a number of “Lyme specialty laboratories” have emerged, claiming singular expertise in LD testing. We investigated the degree of interlaboratory variability of several LD serologic tests—whole cell sonicate (WCS) enzyme-linked immunosorbent assay (ELISA), immunoglobulin M (IgM) and immunoglobulin G (IgG) Western blots (WBs), and an ELISA based on the conserved sixth region of variable major protein—like sequence expressed (C6)—that were performed at 1 university laboratory, 1 commercial laboratory, and 2 laboratories that specialize in LD testing.

Methods. Serum samples from 37 patients with posttreatment Lyme syndrome, as well as 40 medically healthy controls without prior LD, were tested independently at the 4 laboratories.
**BORRELIOSES - Lyme Disease**

Lyme Immunoblot IgM Serum

**IGX Criteria:** Positive

**CDC/NYS Criteria:** Positive

<table>
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<tr>
<th>Band (kDa)</th>
<th>23*</th>
<th>31*</th>
<th>34*</th>
<th>39*</th>
<th>41*</th>
<th>93</th>
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<tr>
<td>Intensity</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>+++</td>
<td>-</td>
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Band Intensity: Positive: + to ++++, Indeterminate: Inc, Negative: (-)

**INTERPRETATION**

**IGX CRITERIA**

- 2 or more of the starred bands are present (+): 23*, 31*, 34*, 39*, 41* kDa

**CDC/NYS CRITERIA**

- 2 or more of the following bands are present (+): 23*, 39*, 41* kDa

**LIMITATION:** Bands 31* and 34* kDa are present in Lyme vaccinated patients. False antibodies cross react with the 93 kDa antigen.
Laboratory Testing in Lyme Disease

• A good review on testing is available here:

Future Testing?
Revisiting the Lyme Disease Serodiagnostic Algorithm: the Momentum Gathers

Adriana R. Marques

ABSTRACT Lyme disease is a tick-borne illness caused by *Borrelia (Borrelia) burgdorferi*, and it is the most common vector-borne disease in the United States, with an estimated incidence of 300,000 cases per year. The currently recommended approach for laboratory support of the diagnosis of Lyme disease is a standard two-tiered (STT) algorithm comprised of an enzyme-linked immunoassay (EIA) or immunofluorescence assay (IFA), followed by Western blotting (WB). The STT algorithm has low sensitivity in early infection, and there are drawbacks associated with the WB use in practice. Modified two-tiered (MTT) algorithms have been shown to improve the sensitivity of the testing in early disease while maintaining high specificity. In this issue of the *Journal of Clinical Microbiology*, A. Pegalajar-Jurado et al. (J Clin Microbiol 56:e01943-17, 2018, https://doi.org/10.1128/JCM.01943-17) report the results of their evaluation of the Liaison VIcE CLIA, the Captia *B. burgdorferi* IgG/IgM EIA, and the C6 *B. burgdorferi* (Lyme) EIA as MTT algorithms compared with results with the STT algorithm using the same tests as the first-tier test and the ViraStripE IgM and IgG WBs as the second-tier test. The results showed that all MTT algorithms had higher sensitivities than STT algorithms and were highly specific. These results showed that MTT approaches are a valid alternative to the currently recommended STT algorithm for serodiagnosis of Lyme disease, opening the door for the development of rapid diagnostics and point-of-care testing that can provide diagnostic information during the early stages of the disease.
Diagnosis of Early Localized Lyme Disease

• Predominantly clinical based on erythema migrans
  • Present in ~75% of cases
  • Typically 1-2 weeks after tick exposure (3-30 day range)
Erythema Migrans

“Classic” Lyme disease rash

• Recommended:
  https://www.cdc.gov/lyme/signs_symptoms/rashes.html
Diagnosis of Early Localized Lyme

• Systemic symptoms can occur in patients with single EM as well as in disseminated disease, resembling a viral syndrome without respiratory symptoms.

• 79 patients with EM (14 w/ multiple)
  • 68% had systemic symptoms
    • Fatigue – 54%
    • Arthralgia/Myalgia – 44%
    • Headache – 42%
    • Subjective fever/chills – 39% (documented in 16%)
Diagnosis of Early Localized Lyme

The Clinical Spectrum of Early Lyme Borreliosis in Patients with Culture-confirmed Erythema Migrans

Robert B. Nadelman, MD, John Nowakowski, MD, Gilda Forseter, RN, Neil S. Goldberg, MD, Susan Bittker, MS, Denise Cooper, BS, Maria Aguero-Rosenfeld, MD, Gary P. Wormser, MD, Valhalla, New York

- Fatigue – 54%
- Arthralgia/Myalgia – 44%
- Headache – 42%
- Subjective fever/chills – 39% (documented in 16%)
Diagnosis of Early Localized Lyme Disease

• In the presence of a typical EM rash, laboratory testing is not necessary and can confound the diagnosis
  • Serologic testing is insensitive in early localized disease
  • If serology sent in the presence of a typical EM rash, therapy should **not** be stopped if testing returns negative
Diagnosis of Early Localized Lyme Disease

• If there is diagnostic uncertainty
  • Obtain baseline and follow-up serology 4-6 weeks later
  • Can treat patient empirically based on your clinical suspicion and their preference, or await repeat testing

• Caveats
  • If the patient has known history of Lyme disease or previously positive serology, repeat testing is unlikely to be helpful. You have to decide on treatment without testing.
  • If you make a decision in your office to pursue the testing strategy without treatment, and the initial test returns positive, then don’t wait 6 weeks to treat.
Treatment

• Overview
  • PO doxycycline is the treatment of choice in most cases
    • IV ceftriaxone can be used for a limited number of indications
  • Short courses of therapy are the standard of care
Treatment of Early Localized Lyme

- Treatment is PO
- 1 of 3 antibiotic regimens is recommended
  - Doxycycline 100mg BID
    - Note: doxycycline also treats anaplasma (others do not)
  - Amoxicillin 500mg TID
  - Cefuroxime 500mg BID
- Duration
  - 14-21 days (10 days is effective with doxycycline)
Treatment of Early Localized Lyme

• Evidence for good clinical outcomes

• Notable studies

• Luger et al.. 1995: 232 subjects, doxy vs cefuroxime
  • Success or improvement in
    • 95% of doxycycline treated patients
    • 90% of cefuroxime treated patients
Treatment of Early Localized Lyme

Comparison of Cefuroxime Axetil and Doxycycline in Treatment of Patients with Early Lyme Disease Associated with Erythema Migrans

STEVEN W. LUGER,¹ PHILIP PAPARONE,² GARY P. WORMSER,³ ROBERT B. NADELMAN,³ EDGAR GRUNWALDT,⁴ GEMA GOMEZ,⁵ MICHAEL WISNIEWSKI,⁵ AND JEFFREY J. COLLINS⁵*  

Old Lyme Family Practice, Old Lyme, Connecticut¹; Lyme Disease Center for South Jersey, Absecon, New Jersey²; Division of Infectious Diseases, Department of Medicine, New York Medical College, Westchester County Medical Center, Valhalla, New York³; Shelter Island, New York⁴; and Glaxo Inc., Research Triangle Park, North Carolina⁵
Treatment of Early Localized Lyme

• Evidence for good clinical outcomes

• Notable studies
  • Wormser et al. 2003: 180 subjects
    • 60 received IV CTX x1 + 10 days PO doxycycline
    • 61 received 10 days PO doxycycline
    • 59 received 20 days PO doxycycline
  • No significant difference in outcomes at 20 days, 3 months, 12 months or 30 months
Treatment of Early Localized Lyme

- Evidence for good clinical outcomes
- Notable studies

**Annals of Internal Medicine**

**Duration of Antibiotic Therapy for Early Lyme Disease**

A Randomized, Double-Blind, Placebo-Controlled Trial

Gary P. Wormser, MD; Roshan Ramanathan, MD, MPH; John Nowakowski, MD; Donna McKenna, RN, ANP; Diane Holmgren, RN; Paul Visintainer, PhD; Rhea Dornbush, PhD; Brij Singh, MD; and Robert B. Nadelman, MD
Treatment of Early Localized Lyme

• Evidence for good clinical outcomes

• Notable studies
  • Kowalski et al.. 2010
  • Retrospective study of 607 patients with early Lyme
  • 93% treated with doxycycline
  • Outcomes: “treatment failure-free” at 2 years
    • \( \leq 10 \) days of antibiotic: 99%
    • 11-15 days of antibiotic: 98.9%
    • \( \geq 16 \) days of antibiotic 99.2 %
Treatment of Early Localized Lyme

Antibiotic Treatment Duration and Long-Term Outcomes of Patients with Early Lyme Disease from a Lyme Disease–Hyperendemic Area

Todd J. Kowalski,† Sujatha Tata,§ Wendy Berth,∥ Michelle A. Mathiaso,∥ and William A. Agger†

†Section of Infectious Disease and Departments of §Medical Education and ∥Research, Gundersen Lutheran Medical Foundation, La Crosse, Wisconsin
Conclusion

• Volunteers to present cases
  • Use the case presentation form template

• Please complete evaluation forms for each session
  • CME will be processed once session evaluation form is received at UVM

• UVM Project ECHO materials available at www.vtahec.org

• Please contact us with any questions/concerns/suggestions
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