A Reanalysis of IgM Western Blot Criteria for the Diagnosis of Early Lyme Disease

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A two-step approach for diagnosis of Lyme disease, consisting of an initial EIA followed by a confirmatory Western immunoblot, has been advised by the Centers for Disease Control and Prevention (CDC). However, these criteria do not examine the influence of the prior probability of Lyme disease in a given patient on the predictive value of the tests. By using Bayesian analysis, a mathematical algorithm is proposed that computes the probability that a given patient’s Western blot result represents Lyme disease. Assuming prior probabilities of early Lyme disease of 1%-10%, the current CDC minimum criteria for IgM immunoblot interpretation yield posttest probabilities of 4%-32%. The value of the two-step approach for diagnosis of early Lyme disease may be limited in populations at lower risk of disease or when patients present with atypical signs and symptoms.

In the last several years, Lyme disease has become an increasing public health concern in certain geographic regions within the United States, particularly the Northeast and upper Midwest [1]. Although a screening test for Lyme disease using an EIA has become standard, a high false-positive rate has caused the Centers for Disease Control and Prevention (CDC) to suggest confirmatory testing using a Western blot technique for both IgG and IgM type antibodies [2]. An IgM Western blot is considered positive if 2 of the 3 following bands are present: 24, 39, or 41 kDa. An IgG Western blot is considered positive if 5 of the 10 following bands are present: 18, 21, 28, 30, 39, 41, 45, 58, 66, or 93 kDa [3]. Recent studies have raised concerns about both the sensitivity and specificity of the CDC criteria [4, 5]. In particular, these criteria do not consider the pretest probability of Lyme disease when interpreting test results. By use of Bayesian analysis, this study attempts to establish the predictive value of the CDC criteria for IgM Western blot interpretation when pretest probabilities are considered.

Methods

An anticipated range of pretest probabilities of Lyme disease was estimated from recently published reports from Lyme disease referral centers in areas endemic for Lyme disease [6–9]. The prevalence of active Lyme disease in this setting ranged from 1% in self-referred patients to 40% in physician-referred patients. Even among patients with positive EIA serology, only 6%-34% were confirmed to have active Lyme disease.

The sensitivity and specificity of specific Western blot bands were estimated from a study by Engstrom et al. [10], which was used by the CDC to develop IgM immunoblot criteria. Engstrom et al. assumed that patients tested by Western blot already had a positive IgG or IgM EIA test. The frequency of Western blot bands in patients with Lyme disease is probably enhanced by the last assumption [10, 11]. The controls in the Engstrom study were obtained from non–Lyme-endemic areas. Although the most appropriate controls for this analysis would consist of normal patients with false-positive EIA results, the lack of false-positive EIA results in the Engstrom control group renders this goal unattainable. For the purpose of this analysis, the IgM immunoblot band sensitivities and specificities reported by Engstrom et al. [10] in persons with positive EIAIs will be assumed to be correct.

From a mathematical perspective, the Western blot may be viewed as a series of separate tests performed in parallel, some with positive and others with negative results. The posttest probability that a given immunoblot pattern represents Lyme disease may be computed by the formula:

\[
P(B_i \ldots B_n | L) = \frac{P(B_i \ldots B_n | L) \times P(L)}{P(B_i \ldots B_n | L) \times P(L) + \sum_{i=1}^{n} P(B_i | E) \cdot \sum_{i=1}^{n} P(B_i | E) \times P(E)}
\]

where bands \(B_i \ldots B_n\) are significantly associated with Lyme disease, bands \(B_i \ldots B_n\) represent positive test results, bands \(B_i' \ldots B_n'\) represent negative test results, \(P(L)\) represents the prior probability of Lyme disease, and \(P(E')\) represents the probability of not Lyme disease. The proof of this formula is outlined in Appendix 1. This formula assumes that the false-positive bands observed in controls are independent of each other—an assumption that may be violated in certain disease states. To test the assumption of band independence in healthy controls, the observed frequency of multiple false-positive bands was compared to the frequency predicted by assuming independence. The latter frequency was calculated by standard
probability methods outlined in Appendix 2. The observed frequency was then compared with the expected frequency (extrapolated to the nearest whole number) by \( \chi^2 \) with Yates’s correction (2 × 2 contingency tables, \( P < .05 \)). Data from control groups from other studies were used to further evaluate this independence assumption [4, 12] (unpublished data).

The minimum CDC band criteria for determining a positive IgM immunoblot were used for this analysis (i.e., positive 24- and 41-kDa bands with a negative 39-kDa band). Although I did not know the exact band patterns present in this data set, it can be deduced that the frequency of a given band pattern cannot be more frequent than that of its least frequent component. Since the 39-kDa band was absent in 16% of the persons with Lyme disease studied by Engstrom et al. [10], this value represents an upper bound of the true frequency of the minimum criteria.

Because the sample size in the study by Engstrom et al. [10] was small, additional analysis was undertaken to estimate the degree of error in the posttest probability results. To derive an upper-bound estimate for the posttest probability of a given Western blot band pattern, 90% confidence intervals (CIs) were calculated for the involved variables using either a normal distribution or a binomial distribution, depending on the sample size. The upper-bound estimate for the posttest probability was determined by using the highest band pattern frequency among Lyme disease patients and the lowest band frequencies among controls.

### Results

From the study by Engstrom et al. [10], the sensitivity and specificity of the 24-, 39-, and 41-kDa bands at the time of the initial patient evaluation were 0.840/0.560, 0.840/0.933, and 0.680/0.907, respectively. The predicted frequency of multiple false-positive significant IgM Western blot bands in healthy controls was 7.12%. When these values are rounded to the nearest whole number, one might expect 5 of 75 control patients will be falsely positive. By observation, only 6 (8%) of 75 control subjects had ≥2 significant IgM bands—a result not statistically different from that predicted by assuming band independence.

I evaluated additional data from control groups of Ledue et al. [4] and Dressler et al. [12] from non-Lyme-endemic areas and from the CDC for a Lyme-endemic area (Trevejo RT, unpublished data). From the Ledue study, the 25-, 39-, and 41-kDa IgM bands had specificities of 0.966, 0.966, and 0.793, respectively. From the Trevejo study, the 23-, 39-, and 41-kDa IgM bands had specificities of 0.895, 0.921, and 0.921, respectively. Because monoclonal antibodies to specific band epitopes were not available, the bands identified by Dressler et al. were not directly comparable to the other studies. Therefore, all significant IgM bands identified by Dressler et al. were used to evaluate the independence assumption. The 18-, 21-, 28-, 37-, 41-, 45-, 58-, and 93-kDa bands had specificities of 0.98, 0.96, 1.0, 0.99, 0.98, 0.99, 0.99, and 0.99, respectively. By use of the formula from Appendix 2, the expected-versus-observed frequencies of multiple false-positive IgM bands in controls were derived (see table 1). Although the individual sample sizes were small, collectively these data support the use of the independence assumption for estimating the frequency of false-positive results in normal controls.

The probability that the 24- and 41-kDa bands will occur together in Lyme disease patients without the 39-kDa band is at most 0.16 (90% CI, 0–0.32). The 90% CIs for the 24-, 39-, and 41-kDa band frequencies among controls are 0.358–0.520, 0.026–0.108, and 0.045–0.141, respectively. By use of the above algorithm, the posttest probability of Lyme disease as a function of the pretest probability is illustrated in figure 1. For self-referred persons from areas in which Lyme disease is highly endemic with atypical symptoms (e.g., myalgias and fatigue), the prior probability of Lyme disease may be at most 1%–3% [7, 13]. In this low-risk setting, the posttest probability of Lyme disease may be only 4%–10%, even though both the EIA and Western blot assay are positive (figure 1). Even if one assumes test performance favoring the 90% upper-bound estimate, posttest probabilities of only 18%–36% are generated. Given that 2.8 million Lyme serologic tests are performed annually in the United States and that fewer than 20,000 cases are reported [13], it is likely that most testing is done in a low-risk setting. Assuming the data collected by Engstrom et al. [10] are correct, posttest probabilities of ≥20% are needed to generate posttest probabilities of ≥50%. Even when the most favorable assumptions about test performance are used, it is likely that the pretest probability should be ≥5% to generate posttest probabilities of ≥50%.

Positive EIA serology alone probably does not increase the pre–Western blot probability to >10% in persons with a low pretest risk [6, 13]. Assuming a 10% risk of disease, a minimally positive IgM Western blot result may increase the posttest probability to 32% (figure 1).

### Discussion

Although Western immunoblotting has been advocated as a way to reduce the number of false-positive Lyme EIAs, the current study demonstrates that a significant number of false-positive Western blot results may occur in settings where the prior probability of Lyme disease is ≤10%. These results are similar to those of Sivak et al. [5] and Seltzer and Shapiro [14], who demonstrated that the predictive value of the IgM immunoblot is <50% when the pretest probability of Lyme disease is ≤7%–10%. The concern about false-positive results should focus on persons who are self-referred with atypical signs and symptoms.
Figure 1. Predictive value of IgM immunoblot. Data from [10].

symptoms or who live in areas with a low prevalence of Lyme disease.

There are several reasons why the current analysis may actually overestimate the predictive value of the IgM Western blot when the minimum CDC interpretive criteria are used. The frequency of the band pattern used for this analysis in persons with Lyme disease may be a high estimate, as discussed above. A lower band pattern frequency in Lyme disease patients would result in lower posttest predictive values. It is possible that the frequency of false-positive Western blot bands in normal controls may be higher than stated if the control groups included persons with false-positive EIA results, leading to more frequent false-positive combinations. Theoretically, the false-positive rate of the two-step method may be higher than expected in the populations being tested for Lyme disease due to the presence of cross-reacting conditions, such as connective tissue disorders, syphilis, or viral illnesses [4, 10]. Persons with previously treated or clinically inapparent Lyme disease may have positive serology that produce high posttest probabilities, contributing to lower predictive values in Lyme-endemic areas. Ideally, an accurate pretest assessment would assign such patients low pretest probabilities, impeding testing and thereby limiting false-positive results. Other researchers, recognizing the public’s desire for testing, suggest using controls from endemic areas to improve test interpretation [5]. Although the current analysis suggests that the bands that occur in normal controls are not independent, then they may occur together more frequently than predicted, lowering the predictive value of the blot.

This analysis did not evaluate the predictive value of the Western blot when >3 IgM bands are positive—a setting in which the specificity of the results would likely be higher. Additional data are needed to accurately calculate the predictive value of the Western blot in this setting.

It may be possible to improve the IgM immunoblot by considering the pretest probability of Lyme disease when interpreting the results. One might consider reporting a probability range for a given blot result based on a low (1%–3%), moderate (10%), or high (40%) pretest risk. Higher predictive values may be possible if the interpretive criteria include more bands that are highly specific (37-, 45-, 58-, or 93-kDa bands). The clinician may then choose to interpret the test results by using all of the above factors. A posttest probability of >50% may be considered a reasonable treatment threshold [13], although additional prospective studies are needed to better define this threshold. Higher thresholds may be needed to justify the risk and expense of intravenous therapy. If the patient does not respond to treatment, the clinician should question the diagnosis, particularly if it was based primarily on serology rather than on clinical presentation.

Appendix 1

Given: $i, j, k \in J$ of size $n$, but $i \neq k$, $P(L)$ is the prior prob-
ability of Lyme disease, \( P(L) \) is the probability of not Lyme disease, and \( P(B_i/L) \) is the probability of the \( i \)th Western blot band. \( B_i \), given Lyme disease is present; \( B_i \cap B_j \neq \emptyset \), \( P(B_i) \neq 0 \), \( L \cap B_i \neq \emptyset \), and \( P(B_i/L) \) is significantly greater than \( P(B_i/L') \) (\( P \leq .01 \) for each \( j \)).

1. For any given Western blot, observe bands \( B_1, ..., B_n \) are positive and bands \( B_{i+1}, ..., B_j \) are negative. Then by Bayes theorem [15], \( P(L/B_1...B_{i+1}...B_j) = [P(B_1...B_{i+1}...B_j/L) \times P(L)] / [P(B_1...B_{i+1}...B_j)] \);
2. By standard set theory [15], \( P(B_1...B_{i+1}...B_j) = P(B_1...B_{i+1}...B_j/L) + P(B_1...B_{i+1}...B_j/L') \);
3. By Bayes theorem, \( P(B_1...B_{i+1}...B_j) = P(B_1...B_{i+1}...B_j/L) \times P(L) + P(B_1...B_{i+1}...B_j/L') \times P(L'); \)
4. For all \( j \in J \), assume that bands \( B_i/L' \) are independent. It then follows that all bands \( B_i/L \) are also independent [15]. Therefore, it follows that

\[
P(B_1...B_i...B_j/L) = \prod_{i=1}^{j} P(B_i/L) \times \prod_{i=j+1}^{n} P(B_i/L).
\]

By substituting the product in equation 4 (above) into equation 3, and then substituting the sum in equation 3 into equation 1, the formula in Methods is derived.

### Appendix 2

Given: \( i, k \in J \) of size \( n \), \( B_i/L \) is the \( i \)th Western blot band given Lyme disease is present, \( B_i/L = B_i \cap L \) when \( i = k \), \( P(B_i) \neq 0 \), \( L \cap B_i \neq \emptyset \), and \( P(B_i/L) \) is significantly greater than \( P(B_i/L') \) (\( P \leq .01 \) for each \( j \)).

If it is assumed that Western blot IgM bands \( B_i/L' \) are independent, then one may compute the expected number of \( \geq 2 \) false-positive IgM bands occurring in controls as follows:

1. The chance that \( \geq 1 \) bands will be positive in controls can be computed as

\[
1 - \prod_{i=1}^{n} [1 - P(B_i/L)].
\]
2. Then the chance that exactly 1 band will be positive in controls will be found by using the formula:

\[
\frac{\sum_{i=1}^{n} P(B_i/L) \times \prod_{j \neq i} [1 - P(B_j/L)]}{[1 - P(B_i/L)]}.
\]

3. Finally, subtracting the value derived from the formula in 2 from the formula in 1 yields the chance that \( \geq 2 \) bands will be seen in controls.

### References