Higher C6 enzyme immunoassay index values correlate with a diagnosis of noncutaneous Lyme disease

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A B S T R A C T

The correlation between the Food and Drug Administration–cleared C6 enzyme immunoassay (EIA) C6 index values and a diagnosis of Lyme disease has not been examined. We used pooled patient-level data from 5 studies of adults and children with Lyme disease and control subjects who were tested with the C6 EIA. We constructed a receiver operating characteristic curve using regression clustered by study and measured the area under the curve (AUC) to examine the accuracy of the C6 index values in differentiating between patients with noncutaneous Lyme disease and control subjects. In the 4821 included patients, the C6 index value had excellent ability to distinguish between patients with noncutaneous Lyme disease and control subjects [AUC 0.99; 95% confidence interval (CI) 0.99–1.00]. An index value cut point of ≥3.0 had a sensitivity of 90.9% (95% CI, 87.8–93.3) and specificity of 99.0% (95% CI, 98.6–99.2%) for Lyme disease.

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1. Introduction

Conventional 2-tiered serologic testing for Lyme disease starts with a sensitive first-tier enzyme immunoassay (EIA). If the first-tier test is reactive (positive or equivocal), the more specific second-tier supplemental immunoblots are performed (Centers for Disease Control and Prevention (CDC), 1995). In both adults and children, the United States Food and Drug Administration (FDA)–cleared C6 EIA first-tier test is comparably sensitive to a whole cell sonicate (WCS) EIA alone but has higher specificity (Branda et al., 2011; Wormser, Schliefer, et al., 2013; Molins et al., 2014; Lipsett et al., 2016). However, as a qualitative test, the C6 EIA alone is less specific than conventional 2-tiered testing with immunoblots, and therefore, its use as a stand-alone test has not been recommended (Branda et al., 2018).

Although EIAs typically produce continuous variable optical density index values, many clinical laboratories report a categorical interpretation of this result (positive, negative, or equivocal) that is used to determine whether a second-tier serologic test will be performed. Previous work has demonstrated that WCS or VlsE EIA index values can be used

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to determine the likelihood of Lyme disease (Lipsett et al., 2015; Zwerink et al., 2018). In 1 study of children from a Lyme disease–endemic area who were being evaluated for potential Lyme disease, a WCS EIA index value ≥3.0 had a positive predictive value for Lyme disease of 99.4% [95% confidence interval (CI) 98.1–99.8%] (Lipsett et al., 2015). However, the test characteristics of the C6 EIA index value for the diagnosis of Lyme disease have not been examined.

To this end, we aggregated patient-level data from several published studies of children and adults undergoing evaluation for Lyme disease, as well as healthy control subjects. We selected studies in which participants were tested with the C6 EIA and, if reactive, supplemental IgM and IgG immunoblots were performed. Our primary aim was to examine whether the C6 EIA index value could assist clinical decision making for patients with potential Lyme disease while awaiting confirmatory immunoblot results by examining the correlation between higher C6 EIA optical density index values and a diagnosis of noncutaneous Lyme disease. Our secondary aims were to determine the correlation between higher C6 EIA optical density index values and the diagnosis of Lyme disease with any manifestation (cutaneous or noncutaneous) as well as the correlation with a positive supplemental immunoblot.

2. Materials and methods

2.1. Study design

We performed a systematic review and reanalysis of published studies to evaluate the performance of the commercially available C6 EIA in the diagnosis of Lyme disease (C6 Borrelia burgdorferi EIA, Immunetics™, Boston, MA). We limited our analysis to studies of U.S. patients of any age published prior to December 31, 2017, in which quantitative C6 index values were generated (Branda et al., 2011; Lipsett et al., 2016; Molins et al., 2014; Lipsett et al., 2016; Nigrovic et al., 2017). Each individual study was approved by the institutional review board (IRB) of the participating institution. The Boston Children’s Hospital IRB deemed this study protocol exempt from additional review.

2.2. Study selection

We searched PubMed, Embase, and Web of Science to identify studies evaluating the performance of the commercially available C6 EIA in the diagnosis of Lyme disease (C6 Borrelia burgdorferi EIA, Immunetics™, Boston, MA). We limited our analysis to studies of U.S. patients of any age published prior to December 31, 2017, in which quantitative C6 index values were generated (Branda et al., 2011, 2013; Lipsett et al., 2016; Molins et al., 2014, 2015; Lipsett et al., 2016; Nigrovic et al., 2017). We contacted corresponding authors to obtain patient-level data. We included 1 eligible study because data were not conveniently available for secondary analysis (Lipsett et al., 2013). We assessed the possibility of publication bias with visual assessment of funnel plot. For all included studies, Lyme disease cases were confirmed by the presence of objective clinical findings, appropriate epidemiologic risk, and often with a positive B. burgdorferi culture or PCR result from relevant tissue or fluid samples, and/or with a positive result using 2-tiered serologic testing.

One of the pediatric studies reported results of Lyme disease tests by sample rather than by patient (Lipsett et al., 2016), raising the possibility that a few children could have been included more than once in our analysis if individuals were tested multiple times over the study period. In addition to the published results, we also included unpublished pediatric data provided by this study’s principal investigator (LEN): additional C6 EIA results generated using 690 normally discarded serum samples from a single center as well as 1540 samples from subjects prospectively enrolled in the ongoing Pedi Lyme Net cohort (Nigrovic et al., 2017 http://www.childrenshospital.org/research/centers-departmental-programs/pedi-lyme-net).

2.3. Laboratory testing for Lyme disease

In all cases, 2-tiered serology consisted of a C6 EIA followed by a supplemental immunoblot for samples with a positive or equivocal C6 EIA. All the C6 EIA results were obtained using the same commercially available diagnostic test kit. As recommended by the manufacturer, the testing laboratory converted Lyme C6 EIA optical density index values to “index values” by dividing by a standardized factor. We used cut points recommended by the assay manufacturer to classify C6 EIA index values as negative (optical density values <0.90), equivocal (optical density index values 0.90–1.09), or positive (optical density index values ≥1.10) (Immunetics websites, 2015). IgG and IgM B. burgdorferi immunoblots were each interpreted using standard criteria (Centers for Disease Control and Prevention (CDC), 1995) by the clinical or research laboratory performing the test. Because patients with Lyme disease who have been symptomatic for more than a month should have a B. burgdorferi IgG antibody response (Lantos et al., 2016; Seriburi et al., 2012), we classified patients as seronegative if they had a positive IgM immunoblot alone and had more than 30 days of symptoms (Centers for Disease Control and Prevention (CDC), 1995).

2.4. Data collection

We abstracted the following from the published manuscripts: age range of patients and total number of patients tested. We then contacted the corresponding authors to request the following patient-level data: nature and duration of clinical symptoms, and C6 EIA quantitative optical density index value along with IgM and IgG immunoblot results (when performed). We excluded test results obtained from patients without available data on duration of clinical signs and symptoms.

2.5. Lyme disease diagnosis

We defined a case of Lyme disease with either an EM skin lesion or positive 2-tiered serology in a patient with compatible symptoms (Nigrovic et al., 2017). Reviewed studies defined EM as erythematous skin lesion measuring at least 5 cm in diameter that expands over a period of days to weeks to form a large round lesion, often with partial central clearing. Extracutaneous Lyme disease was divided into the following stages: early disseminated (e.g., cranial neuritis, meningitis, carditis) and late (arthritis). Symptomatic patients without any cutaneous manifestations and negative 2-tiered Lyme disease serology as well as all asymptomatic control patients regardless of 2-tiered results were classified as not having Lyme disease.

2.6. Statistical analysis

Our primary goal was to examine the ability of the C6 EIA index value to predict a diagnosis of noncutaneous Lyme disease. For this analysis, we excluded patients with a diagnosis of single or multiple EM since this is the only manifestation of Lyme disease that can be diagnosed solely using clinical criteria without the need for diagnostic test results. Our secondary goals were to examine the ability of a C6 EIA index value to predict (1) a clinical diagnosis of Lyme disease, regardless of the manifestations (cutaneous or non-cutaneous), and (2) a positive supplemental Lyme disease immunoblot for patients who had an immunoblot performed.

To this end, we plotted the true-positive rate (sensitivity) vs. the false-positive rate (1 − specificity) on a receiver operating characteristic (ROC) curve using regression with clustering by study to adjust for differences. We used the area under the curve (AUC) to measure the C6 EIA index value’s ability to distinguish between samples obtained from individuals from each of the selected groups. We interpreted the AUC using published standards for diagnostic accuracy: AUCs <0.7, poor discriminatory value; AUCs 0.7–0.8, minimally accurate; AUCs
0.8–0.9, good accuracy; or AUCs >0.9, excellent accuracy (Bonsu and

For patients with noncutaneous manifestations of Lyme disease, we
selected the optimal C6 EIA index value cut point for discriminating be-
tween patients with and without Lyme disease. We selected the C6
index value associated with the point where the ROC “curve turns the
corner,” at which every incremental gain in sensitivity results in a sub-
stantial loss of specificity rounded to the closest integer for ease of ap-
lication. For comparison, we examined the performance characteristics
(sensitivity, specificity) of the dichotomized C6 index value for the diag-
nosis of Lyme disease across a range of C6 cut points for all patients. Last,
we repeated the ROC curve analysis for 2 a priori selected subgroups: 1) all
patients regardless of clinical presentation and 2) all patients
who had an immunoblot performed.

We utilized SPSS version 23.0 for all statistical analyses (IBM SPSS
Software; Armonk, NY).

3. Results

We included C6 EIA results from 4 published studies of well-
characterized patients with Lyme disease and control subjects (Branda
et al., 2011; Lipsett et al., 2016; Molins et al., 2014, 2015), plus additional
unpublished data (Nigrovic et al., 2017), for a total of 5135 C6 EIA test
results (Table 1). Using the funnel plot, we did not detect substantial
publication bias (results not shown). Of the included C6 EIA results,
1994 (38.8%) were obtained from asymptomatic control subjects. Of
the eligible C6 assay results, 589 (11.5%) were positive with an index
value ≥1.10, and 69 (1.3%) were equivocal with index values between
0.90 and 1.00.

Of the 5135 included samples, 754 (14.7%) were obtained from pa-
patients with Lyme disease. Among these patients, 315 (41.8%) had cuta-
neous Lyme disease and 439 (58.2%) noncutaneous Lyme disease; 148
patients with Lyme disease. Among these patients, 315 (41.8%) had cuta-
neous Lyme disease and 439 (58.2%) noncutaneous Lyme disease; 148

Fig. 1. The ROC for C6 EIA index value for Lyme disease after exclusion of cutaneous Lyme
disease cases with specific C6 index value cut points indicated (boxes).

Table 1
Included studies.

<table>
<thead>
<tr>
<th>First author</th>
<th>Journal (year)</th>
<th>Overall N = 5135</th>
<th>Patient ages</th>
<th>Noncutaneous Lyme N = 439</th>
<th>Cutaneous Lyme N = 315</th>
<th>Control subjects N = 4381</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branda et al., 2011</td>
<td><em>Clin Infect Dis</em> (2011)</td>
<td>1391</td>
<td>Adults</td>
<td>28</td>
<td>63</td>
<td>Asymptomatic (n = 1300)</td>
</tr>
<tr>
<td>Lipsett et al., 2016</td>
<td><em>Clin Infect Dis</em> (2015)</td>
<td>1634*</td>
<td>Children¹</td>
<td>149</td>
<td>21</td>
<td>Symptomatic (n = 1277)</td>
</tr>
<tr>
<td>Molins et al., 2014</td>
<td><em>Clin Infect Dis</em> (2015)</td>
<td>139</td>
<td>Adults</td>
<td>0</td>
<td>139</td>
<td>Asymptomatic (n = 187)</td>
</tr>
<tr>
<td>Molins et al., 2015</td>
<td><em>J Clin Microbiol</em> (2016)</td>
<td>431</td>
<td>Adults</td>
<td>30</td>
<td>54</td>
<td>Asymptomatic (n = 144)</td>
</tr>
<tr>
<td>Nigrovic et al., 2017</td>
<td>Pedi Lyme Net (Unpublished)</td>
<td>1540</td>
<td>Children¹</td>
<td>232</td>
<td>38</td>
<td>Asymptomatic (n = 966)</td>
</tr>
</tbody>
</table>

* Includes C6 assay results from 690 pediatric samples not included in the published manuscript.
¹ All patients ≥21 years of age.

In our systematic review of both published and unpublished patient-
level data abstracted from 5 studies, there was a strong positive correla-
tion between C6 EIA index values and a confirmed diagnosis of
noncutaneous Lyme disease. The C6 index value has the potential to in-
form clinical decision making, as higher values are associated with a
higher probability of true disease. In particular, if a patient presents
with signs compatible with noncutaneous Lyme disease and the C6
EIA index value is high (>3.0), clinicians could more confidently make
targeted therapeutic decisions while awaiting supplemental immuno-
blot results.

Previous investigations have examined the C6 EIA as a dichotomous
test: nonreactive (negative) versus reactive (positive or equivocal).
Although the sensitivity of the C6 test alone is high, compared with
standard 2-tiered testing, the specificity of the dichotomous test result

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noncutaneous Lyme disease, Diagnostic Microbiology and Infectious Disease, https://doi.org/10.1016/j.diagmicrobio.2018.12.001
Table 2
Performance of C6 EIA index value by cut point to predict noncutaneous Lyme disease.

<table>
<thead>
<tr>
<th>C6 EIA index value cut point</th>
<th>No. with Lyme disease (N = 439)</th>
<th>No. without Lyme disease (N = 4381) n</th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥0.90</td>
<td>439</td>
<td>219</td>
<td>100% (99.1–100%)</td>
<td>95.0% (94.3–95.6%)</td>
</tr>
<tr>
<td>≥1.10</td>
<td>432</td>
<td>157</td>
<td>98.4% (96.8–99.2)</td>
<td>96.4% (95.8–96.9)</td>
</tr>
<tr>
<td>≥2.0</td>
<td>410</td>
<td>67</td>
<td>93.4% (90.7–95.4)</td>
<td>98.5% (98.1–98.8)</td>
</tr>
<tr>
<td>≥3.0</td>
<td>399</td>
<td>45</td>
<td>90.9% (87.8–93.3)</td>
<td>99.0% (98.6–99.2)</td>
</tr>
<tr>
<td>≥4.0</td>
<td>349</td>
<td>31</td>
<td>79.3% (75.5–83.9)</td>
<td>99.3% (99.0–99.5)</td>
</tr>
<tr>
<td>≥5.0</td>
<td>270</td>
<td>23</td>
<td>61.5% (56.9–65.9)</td>
<td>99.5% (99.2–99.7)</td>
</tr>
</tbody>
</table>

Fig. 2. The ROC for C6 EIA index value for all Lyme disease cases with specific C6 index value cut points indicated.
study patients for other emerging Borrelia infections (e.g., *B. miyamotoi*), and the C6 EIA may also be positive in serum samples collected from patients with these infections (Molloy et al., 2018). Fifth, the included Lyme disease diagnostic tests were performed in a variety of clinical and research laboratories. However, this mimics the real-world situation of clinical Lyme disease testing. Importantly, all studies utilized the same FDA-cleared C6 EIA diagnostic kit, and all supplemental immunoblots were performed and interpreted in 1 of 3 large commercial laboratories using standardized interpretative criteria (Centers for Disease Control and Prevention (CDC), 1995). Sixth, standard 2-tier serologic testing has well-recognized limitations that include both false positives and negatives. Although we did not knowingly include patients with previous Lyme disease, a *B. burgdorferi* antibody response can persist for years or even decades even following effective antimicrobial therapy (Kalish et al., 2001). For our analysis, we assume that patients with positive 2-tiered serology and compatible symptoms had active Lyme disease. As we included only a single Lyme disease test at a single point in time, some study patients with early Lyme disease may have had falsely negative 2-tiered serology. In practice, clinicians should consider repeating Lyme disease testing after a few weeks for patients with high clinical suspicion for Lyme disease and a negative initial Lyme disease test (Kaiser, 2000). Lastly, we did not compare the performance of the C6 EIA index value to that of other first-tier EIA tests (e.g., WCS EIA) (Lipsett et al., 2015), so we cannot comment on the relative accuracy.

5. Conclusions

C6 EIA index values can provide valuable, actionable information to guide initial clinician decision making for children or adults with possible Lyme disease. Clinical laboratories should consider reporting C6 EIA index values, along with annotations to aid interpretation and suggest a course of action to the treating clinician. The C6 EIA index value could be factored in along with clinical signs and epidemiological risk factors and, in some cases, could justify targeted initial therapy while avoiding potentially harmful interventions directed toward other entities before the results of supplemental immunoblots become available. Future prospective studies should measure the impact of providing clinicians quantitative index values with interpretation on the care of patients being evaluated for Lyme disease.

Acknowledgment

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References


