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Comparative Cost-Effectiveness of Two-Tiered Testing Strategies for Serodiagnosis of Lyme Disease with Noncutaneous Manifestations

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The mainstay of laboratory diagnosis for Lyme disease is two-tiered serological testing, in which a reactive first-tier enzyme-linked immunosorbent assay (ELISA) or an immunofluorescence assay is supplemented by separate IgM and IgG immunoblots. Recent data suggest that the C6 ELISA can be substituted for immunoblots without a reduction in either sensitivity or specificity. In this study, the costs of 4 different two-tiered testing strategies for Lyme disease were compared using the median charges for these tests at 6 commercial diagnostic laboratories in 2012. The study found that a whole-cell sonicate ELISA followed by the C6 ELISA was the most cost-effective two-tiered testing strategy for Lyme disease with acute-phase serum samples. We conclude that the C6 ELISA can substitute for immunoblots in the two-tiered testing protocol for Lyme disease without a loss of sensitivity or specificity and is less expensive.

Lyme disease is the most frequently reported vector-borne infection in the United States, with over 33,000 confirmed or probable cases reported in 2011 (1). Moreover, the number of cases may be closer to 300,000 when the estimated number of unreported cases is considered (P. Mead, presented at the 13th International Conference on Lyme Borreliosis and Other Tick-Borne Diseases, Boston, MA, 18 August 2013). The most common clinical manifestation is a skin lesion called erythema migrans (2). Noncutaneous manifestations occur later in the course of infection and include cranial nerve palsy, meningitis, myocarditis, and arthritis. An assessment by the Centers for Disease Control and Prevention (CDC) of the clinical features of over 150,000 cases of Lyme disease reported in 1992 to 2006 found that noncutaneous manifestations accounted for over 30% of cases (2). In the United States, serological testing is the mainstay of laboratory diagnosis when noncutaneous manifestations of Lyme disease are present. Two-tiered serological testing, consisting of a first-tier assay (such as an enzyme-linked immunosorbent assay [ELISA] or an immunofluorescence assay) followed by supplemental separate IgM and IgG immunoblots, has been the standard of practice since 1995 (3). Two-tiered testing was implemented because many first-tier assays had relatively poor specificity, with false-positive rates of ≥5% (3, 4). It has been estimated that at least 3.4 million serological tests for Lyme disease are performed annually, which reinforces the need for accurate and cost-effective tests (4, 5).

Although the reported specificity of two-tiered testing for Lyme disease has been as high as 99.5% when testing is performed at academic medical centers (6, 7), in clinical practice the second-tier immunoblot assays have had a number of substantive shortcomings. Immunoblots are more labor-intensive to perform than ELISAs, and interpretation is subjective. In clinical practice, a noteworthy problem has been overinterpretation of weak bands on IgM immunoblots, leading to false-positive results and consequently incorrect diagnoses and unnecessary antibiotic treatment (8). Recognizing the limitations of standard two-tiered testing, Branda et al. (6) evaluated the sensitivity and specificity of a novel two-tiered testing strategy consisting of a conventional first-tier ELISA using a whole-cell sonicate (WCS) of *Borrelia burgdorferi* as the antigen target and supplemental testing of reactive serum specimens using a second ELISA, in which the C6 peptide serves as the antigen target, instead of immunoblotting. The C6 peptide is a highly conserved, 25-amino acid peptide derived from the sixth invariant region of the variable major protein-like sequence, expressed (VlsE), protein (7, 9) of *B. burgdorferi*. The VlsE protein is poorly expressed during *in vitro* culture and therefore appears to be represented minimally or not at all in WCS preparations of *B. burgdorferi* (10, 11). In the study by Branda et al. (6), the WCS ELISA-C6 ELISA two-tiered testing strategy was associated with 100% sensitivity among 28 patients with noncutaneous manifestations of Lyme disease, compared with 78.6% sensitivity for conventional two-tiered testing. In addition, the specificity of the WCS ELISA-C6 ELISA two-tiered testing algorithm was 99.5% when applied to 1,300 control sera, identical to that of conventional two-tiered testing (6).

The purpose of the present study was to compare the cost-effectiveness of four different two-tiered testing strategies, using data on the sensitivity and specificity of the individual two-tiered testing strategies from a large multicenter study conducted in the United States (7). Although single-tiered testing using a C6 peptide ELISA alone has also been proposed, the cost-effectiveness of this approach was not considered in this study because it is less specific than two-tiered testing (6, 7).

MATERIALS AND METHODS

Four two-tiered testing strategies were compared, using sensitivity and specificity values either explicitly stated in a published report by Wormser et al. (7) or subsequently calculated from the same study data. In the study by Wormser et al. (7), the following assays were used: the C6 Lyme ELISA kit (Immunetics, Inc., Boston, MA) plus either of two WCS ELISAs, i.e., the Wampole IgG/IgM ELISA kit (Alere, Inc., Waltham, MA) or the Vidas

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TABLE 1 Direct costs of Lyme disease serological tests, based on a 2012 survey of six diagnostic laboratories

Test[a]	Cost ($)[b]		
	Median	Minimum	Maximum
WCS ELISA	127	110	151
C6 ELISA	180	80	235
Immunoblots (IgM and IgG)	264	234	497
WCS ELISA plus immunoblots	399	310	607
C6 ELISA plus immunoblots	432	314	707
WCS ELISA plus C6 ELISA	309	199	370

[a] WCS, whole-cell sonicate; ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin M; IgG, immunoglobulin G.
[b] C6 ELISA costs were based on available data from 5 diagnostic laboratories, whereas the costs of the other tests listed were based on data from 6 diagnostic laboratories. For two-tiered test combinations, the indicated costs reflect those incurred when both tests are performed at the same diagnostic laboratory.

II Lyme IgG/IgM screening kit (bioMérieux SA, Marcy l'Etoile, France); the immunoblot assays used were the Lyme IgG and IgM immunoblot kits from MarDx/Trinity Biotech (Bray, Ireland).

The estimated cost of each two-tiered serodiagnostic strategy was based on written responses received from six commercial diagnostic laboratories that offer this testing. Inquiries to these laboratories about the undiscounted charges that a patient would incur for a WCS ELISA, for the C6 ELISA, and for IgM and IgG immunoblots for detection of antibodies to *B. burgdorferi* were made during the summer of 2012.

RESULTS

Based on a survey of six commercial diagnostic laboratories, the median charge for a WCS ELISA to detect *B. burgdorferi* antibodies was $127, that for the C6 ELISA was $180 ($n = 5$ laboratories for the C6 ELISA), and that for IgM and IgG immunoblots was $264 (Table 1). Some of these laboratories also offer testing consisting of a first-tier ELISA with subsequent immunoblotting that is cheaper than ordering each of the tests individually. In such a situation, however, the cost associated with a first-tier ELISA with negative results that would not lead to immunoblotting is greater than the cost of an ELISA alone. For this analysis, only the costs of the individual tests were considered. The median charge for the combination of the WCS ELISA and immunoblot testing ordered as individual tests at the same laboratory was $399; similarly, the median charge for the combination of the C6 ELISA and immunoblot testing at the same laboratory was $432, and the median charge for the combination of the WCS ELISA and the C6 ELISA at the same laboratory was $309 (Table 1). For this analysis, cost estimates of two-tiered testing were based on the median costs of the individual tests added together, irrespective of laboratory. This was justified since these cost estimates were similar to the median costs incurred when both tests were performed by the same laboratory.

We also determined the net cost to a patient care center (e.g., clinic or hospital) for each of the four two-tiered testing strategies, by subtracting potential reimbursement from the median charges listed above. For outpatient testing performed at a commercial laboratory, patient care centers directly pay the full negotiated charge for each test and then bill the patient's insurance, a Medicare administrative contractor, a state Medicaid agency, or the patient himself or herself to recover the cost (or a portion of it). Reimbursement rates for a given test vary considerably depending on the payer and the state. For this analysis, reimbursement rates were obtained from the Centers for Medicare and Medicaid Services (CMS) 2012

TABLE 2 Net costs of Lyme disease serological tests

Test[a]	Direct cost ($)[b]	CPT[c] code(s)	Reimbursement ($)[d]	Net cost to care center ($)[e]
WCS ELISA	127	86618	33	94
C6 ELISA	180	86618	33	147
Immunoblots (IgM and IgG)	264	86617 (×2)	59	205
WCS ELISA plus immunoblots	399	86618, 86617 (×2)	92	307
C6 ELISA plus immunoblots	432	86618, 86617 (×2)	92	340
WCS ELISA plus C6 ELISA	309	86618 (×2)	65	244

[a] WCS, whole-cell sonicate; ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin M; IgG, immunoglobulin G. For two-tiered test combinations, the costs shown reflect those incurred when both tests are performed at the same diagnostic laboratory.
[b] Direct costs reflect the median undiscounted costs of reference laboratory testing, based on available data from 5 laboratories (for the C6 ELISA) or 6 laboratories (for all other tests) collected in 2012.
[c] CPT, Current Procedural Terminology.
[d] Potential reimbursements reflect the median Centers for Medicare and Medicaid Services reimbursement rates for 2012.
[e] Net costs were calculated by subtracting reimbursements from direct costs.

Clinical Laboratory Fee Schedule (http://www.cms.gov/Medicare/Medicare-Fee-for-Service-Payment/ClinicalLabFeeSched/clinlab.html), which defines fee-for-service reimbursement rates for outpatient clinical laboratory services. Specifically, as a representation of typical reimbursement rates, we used the median of the 60th percentile reimbursement amount offered by all Medicare part B carriers in 2012. As shown in Table 2, the net cost of the two-tiered testing combining a WCS ELISA with immunoblot testing was $307, the net cost of the two-tiered testing combining the C6 ELISA with immunoblot testing was $340, and the net cost of the two-tiered testing combining the WCS ELISA with the C6 ELISA was $244.

For every 10,000 noncutaneous Lyme disease cases, we estimated that at least 93% would test positive by the various two-tiered testing strategies, as shown in a large clinical study (Table 3) (7). Based on this assumption, the costs associated with four different two-tiered strategies for testing 10,000 individuals with noncutaneous manifestations of Lyme disease are shown in Table 4. Considering only the direct cost of each testing strategy, the least expensive was a WCS ELISA followed by the C6 ELISA, although the reverse strategy of the C6 ELISA followed by a WCS ELISA had an almost identical cost. Similarly, when the net cost to a patient care center was calculated, taking into account potential reimbursement, the WCS ELISA-C6 ELISA strategy was least expensive and the reverse strategy was only slightly more costly.

Using the data on specificity in the study by Wormser et al. (7) (Table 3), the costs of testing 10,000 patients without Lyme disease using the four different two-tiered algorithms are shown in Table 5. Whether direct or net costs were considered, the least expensive testing strategy was again a WCS ELISA followed by the C6 ELISA. The reverse testing strategy of the C6 ELISA followed by a WCS ELISA was 33.7% more expensive when only expenditures were considered and was 46.5% more expensive in net costs.

DISCUSSION

It has been shown previously that a two-tiered testing strategy for Lyme disease in which a first-tier ELISA is supplemented by both

TABLE 3 Sensitivity and specificity of serological testing strategies for Lyme disease in a published study (7)[a]

Testing strategy[b]	Sensitivity (% [95% CI]) in patients with noncutaneous manifestations ($n = 142$)[c]	Specificity (no. testing negative/no. of control serum samples [% {95% CI}])
WCS ELISA	97.9 (94.0–99.6)	2,102/2,208 (95.2 [94.2–96.1])
C6 ELISA	97.2 (92.9–99.2)	2,184/2,208 (98.9 [98.4–99.3])
Two-tiered testing strategies		
WCS ELISA followed by supplemental immunoblots	93.7 (88.3–97.1)	2,196/2,208 (99.5 [99.1–99.7])
C6 ELISA followed by supplemental immunoblots	93.0 (87.4–96.6)	2,197/2,208 (99.5 [99.1–99.8])
WCS ELISA followed by supplemental C6 ELISA	96.5 (92.0–98.9)	2,197/2,208 (99.5 [99.1–99.8])
C6 ELISA followed by supplemental WCS ELISA	96.5 (92.0–98.9)	2,197/2,208 (99.5 [99.1–99.8])

[a] The 95% confidence intervals (CIs) were calculated with the exact method.
[b] WCS, whole-cell sonicate; ELISA, enzyme-linked immunosorbent assay.
[c] Only results obtained from acute-phase serum samples are shown.

IgM and IgG immunoblots is over 90% sensitive among patients with noncutaneous manifestations of Lyme disease (Table 3) (6, 7). Although this testing strategy is substantially less sensitive with acute-phase serum samples from patients with erythema migrans (6, 7), serological testing is not usually needed or recommended in such situations, as the skin lesion is often sufficiently distinctive to permit clinical diagnosis (12).

An alternative two-tiered testing strategy in which a WCS ELISA is supplemented by a C6 ELISA, or the reverse strategy in which a C6 ELISA is supplemented by a WCS ELISA, has been shown to provide sensitivity comparable to or better than that obtained with either of these first-tier ELISAs followed by supplemental immunoblots for Lyme disease patients with noncutaneous manifestations (Table 3) or with erythema migrans (6, 7). Although available data indicate that neither immunoblot testing nor the C6 ELISA is completely independent of the WCS ELISA (6, 13), sequential testing clearly increases specificity (Table 3) (6, 7), as was the original intent of the CDC in the recommendation to adopt the two-tiered testing protocol for Lyme disease (3). A WCS ELISA followed by the C6 ELISA has been shown to be equally specific, at 99.5% in comparison with the WCS ELISA followed by immunoblotting and with the C6 ELISA followed by the WCS ELISA, in two separate studies, each with over 1,000 control serum samples (6, 7).

Among the two-tiered testing strategies considered in this study, the WCS ELISA followed by the C6 ELISA was 27.1% to 44.0% less expensive in direct costs than algorithms that included immunoblots in the second tier for testing patients with noncutaneous manifestations of Lyme disease (Table 4), given the cost assumptions that were made (Tables 1 and 2). When net costs were determined (expenses minus reimbursements), the WCS ELISA-C6 ELISA strategy was 23.9% to 45.5% less expensive. For testing individuals without Lyme disease, two-tiered testing with a WCS ELISA followed by the C6 ELISA was also the least costly of the four strategies evaluated (Table 5), given the cost assumptions made for this analysis (Tables 1 and 2). This was true whether direct expenses were considered alone or net costs were calculated. Of course, regardless of the testing algorithm applied, cost containment is maximized when laboratory testing is avoided for patients for whom the pretest probability of disease is very low.

The cost savings associated with two-tiered testing using a WCS ELISA followed by a C6 ELISA, compared with the other two-tiered testing strategies described herein, can be attributed to several factors. First, as shown here, commercial laboratories typically charge less for a WCS ELISA than for immunoblots and charge more for the C6 ELISA than a WCS ELISA. Second, reimbursement rates are the same for all ELISAs, whether WCS or C6, and, although reimbursement is higher for Lyme immunoblots than for ELISAs, the reimbursement covers only a fraction of the prices charged by commercial laboratories. Thus, it is most cost-effective to combine two ELISAs (without the use of an immunoblot) and to screen patients with the less expensive of them (the WCS ELISA). This strategy may lead to additional cost savings by allowing some patient care centers to perform both elements of a two-tiered testing strategy in-house, rather than involving a commercial laboratory. As demonstrated in a recent national survey

TABLE 4 Estimated costs associated with two-tiered testing strategies for 10,000 patients with noncutaneous manifestations of Lyme disease

Testing strategy[a]	Cost of first-tier testing ($)[b]		Approximate no. of positive first-tier tests[c]	Cost of second-tier testing ($)		Total cost ($)		% greater total cost than least costly approach	
	Direct	Net		Direct	Net	Direct	Net	Direct	Net
WCS ELISA followed by immunoblots	1,270,000	940,000	9,790	2,584,560	2,006,950	3,854,560	2,946,950	27.1	23.9
WCS ELISA followed by C6 ELISA	1,270,000	940,000	9,790	1,762,200	1,439,130	3,032,200	2,379,130	NA[d]	NA
C6 ELISA followed by immunoblots	1,800,000	1,470,000	9,720	2,566,080	1,992,600	4,366,080	3,462,600	44.0	45.5
C6 ELISA followed by WCS ELISA	1,800,000	1,470,000	9,720	1,234,440	913,680	3,034,440	2,383,680	0.07	0.19

[a] WCS, whole-cell sonicate; ELISA, enzyme-linked immunosorbent assay.
[b] Direct costs were calculated from the median undiscounted costs of reference laboratory testing, as listed in Tables 1 and 2. Net costs were calculated by subtracting potential reimbursements from direct costs, as shown in Table 2.
[c] Based on the sensitivity values listed in Table 3.
[d] NA, not applicable.

TABLE 5 Estimated costs associated with two-tiered testing strategies for 10,000 patients without Lyme disease

Testing strategy[a]	Cost of first-tier testing ($)[b]		Approximate no. of positive first-tier tests[c]	Cost of second-tier testing ($)		Total cost ($)		% greater total cost than least costly approach	
	Direct	Net		Direct	Net	Direct	Net	Direct	Net
WCS ELISA followed by immunoblots	1,270,000	940,000	480	126,720	98,400	1,396,720	1,038,400	3.0	2.8
WCS ELISA followed by C6 ELISA	1,270,000	940,000	480	86,400	70,560	1,356,400	1,010,560	NA[d]	NA
C6 ELISA followed by immunoblots	1,800,000	1,470,000	110	29,040	22,550	1,829,040	1,492,550	34.8	47.7
C6 ELISA followed by WCS ELISA	1,800,000	1,470,000	110	13,970	10,340	1,813,970	1,480,340	33.7	46.5

[a] WCS, whole-cell sonicate; ELISA, enzyme-linked immunosorbent assay.
[b] Direct costs were calculated from the median undiscounted costs of reference laboratory testing, as listed in Tables 1 and 2. Net costs were calculated by subtracting potential reimbursements from direct costs, as shown in Table 2.
[c] Based on the specificity values listed in Table 3.
[d] NA, not applicable.

administered by the College of American Pathologists (CAP), fewer than 25% of U.S. clinical laboratories that perform Lyme ELISAs in-house also perform Lyme immunoblots in-house (14). By converting to a testing strategy based on two ELISAs, without an immunoblot component, many clinical laboratories would gain the capacity to perform both tiers of a two-tiered serodiagnostic algorithm for Lyme disease. This would likely provide additional cost savings, because in general it is less expensive to in-source testing than to engage commercial laboratories, assuming that the test volume is sufficient to justify in-house serodiagnostic testing for Lyme disease.

A limitation of this study is that we based the cost calculations on a survey of a relatively small number of diagnostic laboratories, using undiscounted charges. Clearly, a markedly different set of cost figures might have altered the relative cost-effectiveness of the four two-tiered testing strategies considered. However, while many patient care centers receive a discount on the list price from commercial laboratories, the proportional discount typically remains consistent for all tests on the menu. For example, we verified that the percentage of list price paid at one author's institution (Massachusetts General Hospital) was the same for the C6 peptide ELISA as it was for immunoblotting. Thus, although the prices paid for the various tests described herein would vary from institution to institution, based on the discount offered, the cost of one test relative to another should remain fairly consistent and our findings and conclusions should be generally applicable. It should also be noted that our cost analysis applies to reference laboratory testing, and we did not attempt to determine or to compare the costs of the various test kits for users who procure the reagents for in-house testing. Another potential limitation is that the specificity of the first-tier WCS ELISAs in this study was relatively high in comparison with other WCS ELISAs (15). According to the recent CAP survey findings, however, the two WCS ELISAs used in this study were the most commonly used polyvalent WCS assays among participants (14). Nonetheless, if the WCS ELISAs had been assumed to have lower specificity, then the cost of testing would have increased in each of the two-tiered testing protocols in which the WCS ELISA served as the first-tier assay, especially for patients without Lyme disease (who represent the majority of patients who are tested). Finally, in determining the cost-effectiveness of various two-tiered testing strategies for Lyme disease, we limited our analysis to the costs of the tests themselves. We did not attempt to estimate downstream costs related to antimicrobial therapy, other medical care, or adverse outcomes traceable to decisions made on the basis of test results generated by the different two-tiered algorithms.

The results of this cost-effectiveness analysis, in combination with the sensitivity and specificity performance of a WCS ELISA supplemented by the C6 ELISA from two separate studies (6, 7), suggest that consideration should be given to wider adoption of this approach. Complete elimination of the option of ordering supplemental immunoblots is not advocated, however, since neither a WCS ELISA nor the C6 ELISA can provide information on the presence of an expanded IgG response specifically, which is a serological prerequisite for the diagnosis of late Lyme disease (7).

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