Clinical Microbiology and Infection 25 (2019) 1233–1238

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Lyme disease overdiagnosis in a large healthcare system: a population-based, retrospective study

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ARTICLE INFO

Article history:
Received 10 January 2019
Received in revised form
14 February 2019
Accepted 15 February 2019
Available online 22 February 2019

Editor: C. Pulcini

Keywords:
Borrelia burgdorferi
Clinical laboratory techniques
Epidemiology
False-positive reactions
Lyme disease
Medical overuse
Predictive value of tests
Tick-borne diseases

ABSTRACT

Objectives: To evaluate the impact of false-positive IgM immunoblots on Lyme disease treatment and case reporting in a large healthcare system.
Methods: We obtained the results of all Lyme disease serological tests ordered at U.S. Air Force healthcare facilities in the USA between January 2013 and December 2017. We conducted chart reviews to adjudicate positive IgM immunoblots (from two-tier and independent testing) as true positives or false positives using established criteria, and we assessed whether these cases were reported to the U.S. Department of Defense surveillance system.
Results: Of the 18 410 serum tests (17 058 immunoassays and 1352 immunoblots) performed on 15 928 unique individuals, 249/1352 (18.4%) IgM immunoblots were positive. After excluding repeat tests, insufficiently documented cases, and participants with a history of Lyme disease, 212 positive IgM immunoblot cases were assessed. A total of 113/212 (53.3%) were determined to be false positives. Antibiotics were prescribed for Lyme disease for 97/99 (98.0%) participants with a true-positive test and 91/113 (80.5%) participants with a false-positive test. The number of false-positive cases reported to the surveillance system was identical to the number of unreported true-positive cases ($n = 44$).
Conclusions: Lyme disease serological tests were overused in a large healthcare system, and positive results were frequently misinterpreted, leading to misdiagnosis and widespread antibiotic misuse. Underreporting of true-positive cases was offset by overreporting of false-positive cases, suggesting that the discrepancy between the reported incidence and true incidence of Lyme disease may not be as significant as previously assumed. **B.J. Webber, Clin Microbiol Infect 2019;25:1233**
Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Introduction

Transmitted by ixodid ticks and caused by infection with *Borrelia burgdorferi sensu lato* spirochetes, Lyme disease is the most common tick-borne infection in North America and much of Eurasia [1]. In the USA, Lyme disease cases reported to the National Notifiable Disease Surveillance System [2] and the Department of Defense surveillance system [3,4] approximately doubled in number from 2004 to 2016, and it is assumed that most cases remain unreported [2].

The diagnostic workup of early Lyme disease depends on the patient's history and presentation [5]. Individuals with an exposure risk who present with the characteristic erythema migrans lesion should be empirically treated without confirmatory testing [5,6]. Given the possibility of false-positive results, laboratory testing should also be avoided among individuals without an exposure risk or who are experiencing only non-specific symptoms (e.g. pain and fatigue) [6–9]. Laboratory workup in the USA [5–7] and parts of Europe [10] favours a two-step serological test approach, wherein positive or equivocal first-tier immunoassays are reflexed to second-tier Western immunoblots—the interpretation of which in the USA is guided by IgM and IgG criteria established by consensus opinion at the Second National Conference on Serologic Diagnosis of Lyme Disease [11].

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https://doi.org/10.1016/j.cmi.2019.02.020
1198-743X/Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Inappropriately ordering Lyme disease serological tests or incorrectly interpreting results may lead to unnecessary antibiotic use, exposing patients to potential adverse events and promoting antimicrobial resistance. It may also encourage the attribution of non-specific symptoms to Lyme disease, perpetuating misconceptions concerning chronic [12] and relapsing [13] manifestations. In extreme cases, malignancies have been misdiagnosed as Lyme disease [14], and patients have died from superfluous therapy [15,16].

This study was initiated after two Lyme disease cases were reported to the U.S. Department of Defense surveillance system. Both patients presented with influenza-like illness in areas without vector ticks, and neither had a documented travel history. Despite more plausible diagnoses, both were reported as confirmed Lyme disease. These cases contest the assumption that Lyme disease is underreported to passive surveillance systems [2]. Although overdiagnosis based on false-positive immunoblots has been described in adult [17] and paediatric [18] practices within endemic areas, the prevalence of false positives across a large healthcare system has yet to be assessed. The objective of this study was to determine the clinical and public health impact of Lyme disease overdiagnosis in a large healthcare system that spans endemic and non-endemic areas.

Methods

Study design

This population-based, retrospective study was approved by the Air Force Research Laboratory Institutional Review Board. We obtained all Lyme disease serological tests ordered at U.S. Air Force healthcare facilities in the USA between 1 January 2013 and 31 December 2017. Participants included service members, military retirees and their relatives who accessed healthcare at any of the 63 U.S. Air Force military treatment facilities in the USA, which are scattered through areas that are endemic and non-endemic for Lyme disease. Providers at these facilities are expected to follow U.S. national guidelines when diagnosing and treating Lyme disease [6,11]. Participant age was based on the date of the index test.

Testing assumptions

Because first- and second-tier Lyme disease tests are recorded separately in the Composite Health Care System, we assumed that immunoblots certified within 7 days of an enzyme-linked immunoassay or immunofluorescence assay were reflex tests from the same serum sample. We assumed, further, that all immunoassays and immunoblots were conducted similarly throughout the study period, but slightly different assays and methodology may have been used by the multiple military and commercial laboratories conducting the tests.

Data abstraction

The principal investigator (BJW) conducted a standardized electronic chart review in the Armed Forces Health Longitudinal Technology Application for all participants with a positive serum IgM immunoblot. The following variables were abstracted: age; sex; chief complaint or complaints for healthcare seeking; symptom onset date; laboratory sample collection date; state of residence; documented travel within 30 days of clinical presentation; reported tick bite; and evidence of erythema migrans, acute febrile illness, cranial nerve palsy, carditis and meningitis. These conditions, as well as travel history and tick bite, were assumed to be negative if not indicated in the chart. Erythema migrans could either be documented or described as such in the clinical note. Acute febrile illness was defined as a provider diagnosis of fever or temperature $\geq$38.0°C at clinical presentation. Cranial nerve palsy, carditis and meningitis were restricted to provider diagnoses. Atypical symptoms were defined as complaints not associated with these usual clinical presentations of acute Lyme disease. We verified that at least two of the three diagnostic IgM immunoblot bands (24, 39 and 41 kDa) were recorded as positive [11]. Participants with a documented history of Lyme disease or incomplete electronic health records (i.e. no notes associated with the Lyme disease serology order) were excluded from analysis. For participants with multiple positive IgM immunoblots during the study period, subsequent tests were excluded given the possibility of prolonged seropositivity after successful treatment [19].

Criteria for assessing immunoblots

We used criteria published by Seriburi et al. [17] to adjudicate positive IgM immunoblot cases as true or false positives. A test was considered to be a false positive if at least one of four conditions applied: (1) testing failed to achieve seropositivity criteria, (2) the individual lacked exposure risk, (3) the individual was asymptomatic or reported only non-specific or atypical symptoms, or (4) follow-up serological testing within 30 days of the positive IgM immunoblot was negative. Failure to achieve seropositivity criteria was subcategorized as (1A) first-tier test omitted, (1B) first-tier test negative, (1C) symptoms persisted beyond 30 days with a negative IgG immunoblot, and (1D) IgM immunoblot with fewer than two positive bands [11]. Lacking exposure risk was subcategorized as (2A) testing ordered in December through March and (2B) residence in a state without *Ixodes scapularis* or *Ixodes pacificus* ticks (i.e. Alaska, Arizona, Colorado, Hawaii, Idaho, Montana, Nebraska, Nevada, New Mexico, South Dakota and Wyoming) [20] and no documented travel history within 30 days of presentation.

Public health reporting

Per the U.S. federal policy [21,22], all Lyme disease cases diagnosed at military treatment facilities must be reported to the Department of Defense surveillance system. This system uses the Lyme disease case definition developed by the U.S. Council of State and Territorial Epidemiologists, wherein a combination of clinical, epidemiological and laboratory criteria are used to classify cases as confirmed, probable or suspected [23]. Cases are classified and reported by the local public health unit at each military treatment facility, similar to the process used in civilian jurisdictions. We queried the surveillance system for cases reported between 1 January 2013 and 30 September 2018, allowing for delayed reporting up to 9 months after the study period end date.

Statistical analysis

Descriptive statistics and two-sided Fisher exact tests with 95% CI were used to describe the history and clinical presentation of individuals, to compare false-positive proportions by age (children (aged <18 years) versus adults (aged $\geq$18 years)) and by sex, and to assess the prevalence of true and false positives reported to the surveillance system. Data were analysed using Base SAS 9.4.

Results

Population

In total, 18 410 serum tests (17 058 immunoassays and 1352 immunoblots) were performed on 15 928 unique individuals (8306

female and 7622 male; mean age 40 years (range 3 months to 95 years)) presenting at Air Force healthcare facilities in the USA over the study period. Of the first-tier serological tests, 819/17 058 (4.8%) were positive or equivocal, and of these 753/819 (91.9%) were reflexed to immunoblot testing. An additional 507 immunoblots were performed without first-tier testing, and 92 were performed after a negative screen. Of all immunoblots performed, 315/1352 (23.3%) were positive: IgM only ($n = 178$); IgG only ($n = 66$); IgM and IgG ($n = 71$). Positive IgG only immunoblots ($n = 66$) were excluded, as were positive IgM immunoblots that (i) represented a repeat positive ($n = 21$), (ii) contained insufficient documentation in the chart ($n = 12$), or (iii) occurred in someone previously diagnosed with Lyme disease ($n = 4$), leaving 212 tests for assessment (Fig. 1).

Classifications of true- and false-positive tests

Of the 212 IgM immunoblots included, 113 were adjudicated as false positives and 99 as true positives. False-positive and true-

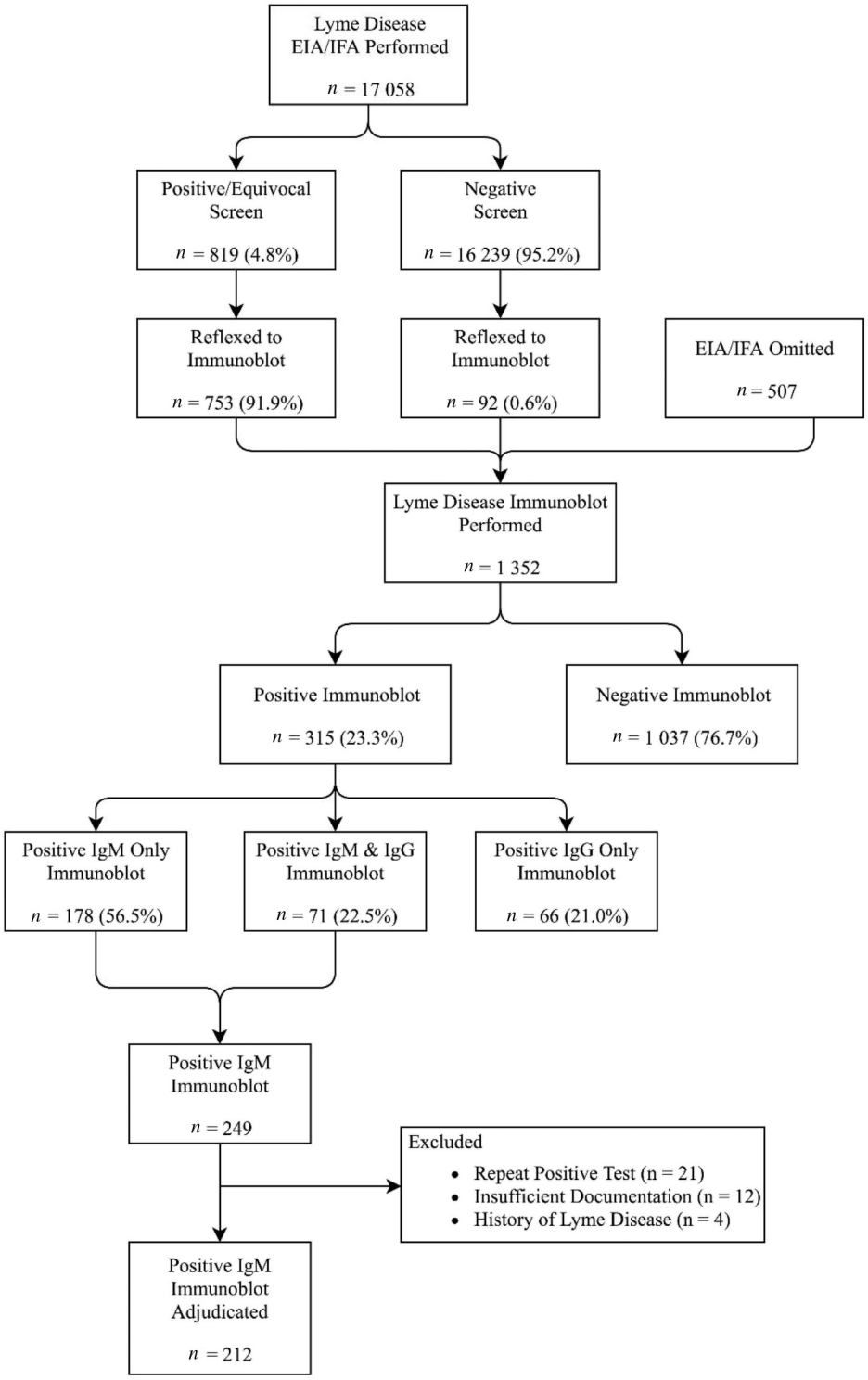

Fig. 1. Lyme disease serological tests ordered at the U.S. Air Force military treatment facilities from 1 January 2013 to 31 December 2017. Abbreviations: EIA, enzyme-linked immunoassay; IFA, immunofluorescence assay.

positive proportions were 53.3% (95% CI 46.3%–60.2%) and 46.7% (95% CI 39.8%–53.7%), respectively. False-positive results met one (33/113; 29.2%), two (54/113; 47.8%), three (22/113; 19.5%), or all four (4/113; 3.5%) criteria (Table 1). If all participants were assumed to have an exposure risk, the true-positive proportion would increase to 47.6% (95% CI 40.8%–54.6%). False-positive proportions were different between adults (95/163; 58.3%) and children (18/49; 36.7%) ($p = 0.008$) and between females (66/100; 66.0%) and males (47/112; 42.0%) ($p < 0.001$).

History and physical examination findings

False-positive IgM immunoblots were identified for individuals residing in Texas (22/113; 19.5%), Maryland (18/113; 15.9%), Virginia (10/113; 8.8%), and 28 other states. True positives occurred in participants who resided in or had recently travelled to 22 states, the most common of which were Maryland (28/99; 28.3%), Virginia (15/99; 15.2%), New York (9/99; 9.1%), New Jersey (7/99; 7.1%), and Pennsylvania (7/99; 7.1%).

Seven participants with false-positive results were asymptomatic at presentation. The chief complaint or complaints of participants with atypical presentation included arthralgia (38/90; 42.2%), fatigue (18/90; 20.0%), rash (15/90; 16.7%), headache (9/90; 10.0%), neuropathy (9/90; 10.0%), skin abscess (7/90; 7.8%), myalgia (6/90; 6.7%), visual changes or ocular pain (5/90; 5.6%), vertigo (4/90; 4.4%), syncope (3/90; 3.3%), and gastrointestinal discomfort (2/90; 2.2%). Participants classified as true positives presented with erythema migrans (62/99; 62.6%), acute febrile illness (56/99; 56.6%), cranial nerve palsy (10/99; 10.1%), carditis (4/99; 4.0%), and meningitis (4/99; 4.0%). A tick bite was reported by 14/113 (12.4%) participants with a false-positive test and 31/99 (31.3%) participants with a true-positive test.

Unnecessary testing

Serological testing was probably unwarranted in 158/212 (74.5%) participants included in the analysis: 104 participants had a low pretest probability of Lyme disease because of atypical presentation or lack of exposure risk, and 54 could have been treated without serological testing because they presented with erythema migrans in the context of exposure risk.

Antibiotic use

Most participants classified as false positives (91/113; 80.5%) received antibiotics for a documented indication of Lyme disease: oral doxycycline alone ($n = 78$); oral amoxicillin alone ($n = 10$); oral cefuroxime, clarithromycin and minocycline ($n = 1$); intravenous ceftriaxone alone ($n = 1$); and oral doxycycline and intravenous ceftriaxone ($n = 1$). Nearly all participants classified as true positives (97/99; 98.0%) received antibiotics: oral doxycycline alone ($n = 81$), oral amoxicillin alone ($n = 12$), intravenous ceftriaxone alone ($n = 2$), and oral doxycycline and intravenous ceftriaxone ($n = 2$).

Case reporting

A total of 55/99 (55.6%) true-positive cases were reported to the U.S. Department of Defense surveillance system, compared with 44/113 (38.9%) false-positives ($p = 0.011$). For the reported cases, 50/55 (90.9%) true positives and 41/44 (93.2%) false positives were classified as confirmed or probable.

Discussion

Of the positive Lyme disease IgM immunoblots obtained in a large U.S. healthcare system, we adjudicated 113/212 (53.3%) as false positives. Assuming all participants had a genuine exposure risk, over half would still be considered false positives by failing to meet seropositivity criteria [11], by presenting asymptomatically or atypically, by having negative follow-up serological testing within 30 days of the positive IgM immunoblot, or by exhibiting a combination of these criteria.

The false-positive percentage in this study exceeds those previously noted in adult (27.5%) [17] and paediatric (28.7%) [18] populations within highly endemic areas. This may be explained by the broader geographic distribution of cases in the present cohort, as serology features a lower positive predictive value in less endemic or non-endemic areas [5,9,13]. Despite its high specificity when used as a second-tier test [19], the Lyme disease IgM immunoblot result should be interpreted in light of the local disease prevalence [5,9,13]. Even in endemic areas, 64/144 (44.4%) clinicians misinterpreted a positive immunoblot result, and 67/144 (46.5%) admitted confusion regarding itemized band results [24]. Laboratories could assist clinicians in two ways: first, by requiring documentation of symptom duration on serology orders and, for patients with symptoms persisting beyond 30 days, reflexing positive or equivocal first-tier tests to the IgG immunoblot alone [5,24]; and second, by changing the format of immunoblot results so that interpretation is more easily distinguished from the individual bands.

Table 1
Assessment of Lyme disease IgM immunoblots ($n = 212$)

False-positive criteria	Meeting criterion, *n* (%)[a]
1. Failure to meet seropositivity criteria	65 (30.7)
A First-tier test omitted	27 (12.7)
B First-tier test negative	8 (3.8)
C Symptoms in excess of 30 days with a negative IgG immunoblot	45 (21.2)
D Immunoblot did not meet band criteria for reactivity [11]	1 (0.5)
2. Lack of exposure risk	52 (24.5)
A Testing performed from December through March	41 (19.3)
B Participant resided exclusively in states without documented *Ixodes scapularis* or *Ixodes pacificus* ticks [20] and had no documented travel history	18 (8.5)
3. Asymptomatic or atypical symptoms at time of testing	97 (45.8)
A Asymptomatic	7 (3.3)
B Symptoms atypical for early Lyme disease[b]	90 (42.5)
4. Negative serology within 30 days of positive test	9 (4.2)

[a] Many of the 113/212 (53.3%) assessed as false positives met multiple criteria. By number of criteria met: one (33/113; 29.2%); two (54/113; 47.8%); three (22/113; 19.5%); four (4/113; 3.5%).
[b] No documented or described erythema migrans lesion, acute febrile illness, cranial nerve palsy, carditis or meningitis.

This study highlights additional concerns regarding laboratory testing for Lyme disease. First, although two-tier testing is considered the standard of care in the USA [6,11] (whereas European guidelines vary according to local epidemiology and microbiology [10]), 599/1352 (44.3%) immunoblots were performed after a negative or omitted immunoassay. Second, the number of positive immunoblots was small relative to the large volume of diagnostic workups. Accounting for both immunoassays and immunoblots performed independently of first-tier tests, 315/17 565 (1.8%) workups resulted in a positive immunoblot (IgM, IgG, or both). As this pre-test probability is far lower than the recommended 10% [10] or 20% [1] for a diagnostic test, the positive predictive value in this population would be far from desirable. Third, serology was not uniformly reproducible, as 9/212 (4.2%) individuals tested seronegative within 30 days of a positive IgM. Of note, discordance was even higher in the study by Seriburi et al., in which 20/50 (40.0%) participants tested seronegative within 4 weeks of a positive IgM [17]. Finally, although this study focused on false-positive IgM immunoblots, overdiagnosis may also occur subsequent to false-positive IgG results [19]; this should be investigated in future research.

To enhance Lyme disease diagnostics on a population level, reducing unnecessary testing is more important than improving laboratory assays. Among the participants with a true-positive IgM immunoblot in our cohort, 54/99 (54.5%) presented with erythema migrans and an exposure risk and therefore could have been diagnosed and treated without laboratory testing [5,6]. Over-reliance on laboratory confirmation was also noted in a large survey of healthcare providers, in which only 373/2000 (18.7%) chose to withhold serological testing in a scenario describing a patient in an endemic area who had classic erythema migrans [25]. Laboratory testing is also unwarranted when patients have not been in an area with vector ticks or present with only atypical symptoms (or asymptomatically) [6–9], which characterized 104/113 (92.0%) of the false-positive IgM immunoblots in this study. Among these, 14/104 (13.5%) participants reported a tick bite, suggesting that the bite alone may have prompted unnecessary testing.

The ubiquity of non-specific symptoms among patients evaluated for Lyme disease has been described previously [13,17,26]. As these symptoms can be chronic and debilitating, clinicians may feel pressured to order Lyme disease serological tests, even in areas of low pre-test probability [26]. In the present study, 91/113 (80.5%) participants with a false-positive IgM immunoblot received antibiotics for Lyme disease, including intravenous ceftriaxone for two individuals, suggesting that antibiotic stewardship should begin with appropriate laboratory testing. Clinicians may find it easier to explain why Lyme disease serological tests are unnecessary in the first place, rather than explaining why antibiotics are unnecessary after obtaining a false-positive result. These discussions can be particularly challenging in the context of prolonged, recurring and atypical symptoms—especially when a patient has already been misdiagnosed with Lyme disease. Advice for clinicians in these situations has been published elsewhere [12].

Estimates of Lyme disease incidence around the world typically assume a large burden of unrecognized cases [2,27]. For example, in light of medical claim [28] and commercial laboratory [29] data, the true incidence of Lyme disease in the USA is thought to be eight-to ten-fold higher than the reported incidence [2]. The present study challenges this assumption. In our cohort, 55/99 (55.6%) true-positive cases and 44/113 (38.9%) false-positive cases were reported to the U.S. Department of Defense surveillance system. Forty-four true-positive cases were not reported, which was offset by the 44 false-positive cases that were reported. Moreover, the vast majority of true positives (50/55; 90.9%) and false positives (41/44; 93.2%) were classified as confirmed or probable cases in the surveillance system, suggesting that false-positives affect the disease incidence reported by the U.S. Centers for Disease Control and Prevention, which excludes suspected cases [2]. Although passive surveillance systems underestimate the incidence of all diseases to the extent persons do not seek care, the discrepancy between the reported incidence and true incidence of Lyme disease may be smaller than assumed because of misinterpretation of IgM immunoblot results by clinicians and public health practitioners. Of note, surveillance systems that rely entirely on laboratory testing would miss cases diagnosed clinically.

Although this study benefits from a large and diverse population in terms of demographic profile and geographic distribution, the findings should be interpreted in light of its limitations. First, as a retrospective study using data abstracted from chart reviews, important variables, such as travel histories and presenting symptoms, may be missing or incomplete in the participants' medical records. Second, individuals without Lyme disease may have been misclassified as true positives. Three participants categorized as true positives received concomitant diagnoses associated with false-positive Lyme disease serology: babesiosis [8], infectious mononucleosis [30], and rheumatoid arthritis [30]. Moreover, cross-sectional serosurveys in highly endemic areas have found background seropositivity as high as 4% [31], suggesting that persons with remote Lyme disease histories may exhibit a prolonged IgM response [1,6]. Third, by incorporating results from multiple laboratories, which may use assays that have different test characteristics, this study does not address the false-positive percentage associated with any particular assay. Fourth, this study assessed a population with access to a healthcare system that does not charge patients for laboratory testing and prescriptions ordered within the network. The findings may not be generalizable to uninsured or underinsured populations.

Overtesting for and overdiagnosis of Lyme disease were common in this large U.S. healthcare system. Clinicians should order serological testing judiciously in accordance with national guidelines. Unnecessary testing and incorrect interpretation of positive IgM immunoblots may squander resources, prompt unwarranted antibiotic use, encourage antimicrobial resistance, and inflate estimates of disease incidence. Reduction of superfluous testing and better assessment of positive IgM immunoblots are the joint responsibility of clinicians, microbiologists and public health personnel.

Contributions of authors

BW contributed to study design, analysis and interpretation of data, statistical analysis and drafting of the manuscript. RB contributed to study design, analysis of data, statistical analysis and drafting of the manuscript. LC contributed to study design, analysis and interpretation of data, critical revision of the manuscript and study supervision. JE contributed to study design, acquisition of data and critical revision of the manuscript. SP contributed to acquisition of data, critical revision of the manuscript and statistical analysis. KG contributed to study design, critical revision of the manuscript and study supervision.

Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Air Force, the Department of Defense, or the U.S. Government.

Transparency declaration

The authors have no conflicts of interest to disclose.

Funding

No outside funding was received.

Acknowledgements

The authors wish to thank Ms Sandy Kawano, technical editor, Aeromedical Research Department, U.S. Air Force School of Aerospace Medicine, for her assistance editing this manuscript. This project was supported in part by an appointment to the Research Participation Program for the U.S. Air Force School of Aerospace Medicine, Public Health and Preventive Medicine Department, administered by the Oak Ridge Institute for Science and Education through an agreement between the U.S. Department of Energy and U.S. Air Force School of Aerospace Medicine, Public Health and Preventive Medicine Department.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2019.02.020.

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