Brief Report

Sex Differences in the Clinical and Serologic Presentation of Early Lyme Disease: Results From a Retrospective Review

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ABSTRACT

Background: Lyme disease is the most common vector-borne disease in the United States, and the number of reported cases has more than doubled between 1992 and 2008. Few studies have explicitly examined sex-based differences in the clinical presentation of or serologic response to early Lyme disease. It is unknown whether the sex-based variability observed in other infectious diseases is relevant to this clinical setting.

Objective: This study retrospectively examined clinical and serologic differences by sex among a community case series of patients with a current or past episode of confirmed early Lyme disease.

Methods: This was a retrospective, consecutive case series of adult patients in Maryland enrolled from August 2002 to August 2007 meeting criteria for a current or past episode of confirmed early Lyme disease. Clinical variables and patients' self-report surrounding illness onset were abstracted through chart review. All serologic tests drawn within 3 months of illness onset were interpreted using Centers for Disease Control and Prevention criteria.

Results: In a total of 125 patients, there were no significant differences in clinical presentation by sex. The initial self-misdiagnosis rates for men and women were 10% and 18%, respectively (P = NS). Among the 62 patients with a serologic test as part of their clinical evaluation, 50% of men had a positive, 2-tier result compared with 32% of women (P = NS). Among the 41 patients with a positive ELISA, median ELISA values (3.4 vs 2.0; P = 0.03) and median number of immunoglobulin G (IgG) bands (4 vs 2; P = 0.03) were significantly higher among men.

Conclusions: In this small, retrospective sample, we found evidence for sex-based differences in the magnitude of ELISA and IgG serologic response to early Lyme disease. Such differences could have implications for appropriate diagnosis, treatment, and disease classification. Larger, prospective studies are needed to replicate the results found in this study and to examine their relationship to sex-based immunologic variability. (*Gend Med.* 2010;7:320–329) © 2010 Excerpta Medica Inc.

Key words: Lyme disease, sex-based differences, serologic testing.

INTRODUCTION

Lyme Disease

Lyme disease, caused by infection with the tick-borne spirochete *Borrelia burgdorferi*, is the most common vector-borne disease in the United States. The number of reported cases per year has more than doubled between 1992 and 2008.[1,2] While almost 29,000 new confirmed cases were reported in 2008,[2] additional studies have reported that the actual number of cases of Lyme disease may exceed reported cases by a factor of 6 to 12 in endemic areas.[3,4] Fifty-three percent of all cases reported to the Centers for Disease Control and Prevention (CDC) between 1992 and 2006 were among men.[1]

The hallmark of early Lyme disease is a localized skin infection, erythema migrans (EM), which occurs at the site of the bite of an infected tick in an estimated 70% to 80% of patients who become infected.[1,5] If untreated, early Lyme disease may progress to later manifestations with neurologic, cardiac, or musculoskeletal involvement. In early disease, a physician-documented EM is diagnostic of Lyme disease and does not require serologic confirmation. Accurate identification of this rash continues to be challenging in community settings,[6] though it remains essential for correct diagnosis because serology is positive in as few as 40% of patients early in infection when initial evaluation is likely to occur.[7] Serologic testing criteria for early Lyme disease rely on a 2-tier approach. Samples positive or equivocal by ELISA are then tested for both immunoglobulin (Ig) M and IgG antibodies by Western blot. Western blots are considered positive if a minimum number of specific bands are found to be present.[8]

Disease Models of Sexual Dimorphism

There is evidence from both infectious and chronic disease models that biological sex is an important variable in biomedical research.[9] The added sociologic effect of gender on illness perception or care-seeking behavior may further compound dissimilarity in disease outcomes.[10] Sex-based differences resulting from genetic, hormonal, and/or environmental factors may contribute to variability in disease pathogenesis, progression, and response to treatment. For example, it has been shown that HIV-1–infected women progress to AIDS at the same rate as men despite lower viral loads, a finding with implications for treatment initiation guidelines.[11,12] Recent studies of cardiovascular disease have shown that relative to men, women generally have a higher risk of heart disease, poorer outcomes, and delayed appropriate clinical interventions.[13,14] Since 1999, the American Heart Association has published separate guidelines for cardiovascular disease prevention in women.[15]

Underlying immunologic response to infection has also been shown to vary by sex.[16–18] For many infectious pathogens, female sex is believed to be protective because of an ability to mount more robust immune responses.[18,19] This factor is also thought to contribute to higher female-to-male ratios in many autoimmune diseases, including lupus and multiple sclerosis.[19] In the development of antibody response to vaccination, higher titers have been shown among females for some pathogens and among males for others.[20] Sex-based differences in both clinical presentation and immunologic response may prove an important factor in understanding variability of disease manifestation and sensitivity of clinical guidelines.

Sex Differences and Early Lyme Disease

Few existing studies have explicitly examined sex-based differences in either the clinical or serologic presentation of patients acutely infected with *B burgdorferi*. One study of 118 patients in Sweden, where different *Borrelia* species are more prevalent, found a significantly longer duration from treatment to EM lesion resolution among women compared with men (11 days vs 7 days, respectively; *P* < 0.001).[21] The same study also found that among those infected with *B afzelii*, females had an 11-fold higher odds (*P* < 0.05) of developing nonannular lesions than did males. Among patients infected with *B burgdorferi* in Sweden, one study suggested that susceptibility to reinfection may vary by gender and menopausal status, resulting from the effect of decreasing estrogen levels on T-helper 1 (Th1) versus Th2 immunity.[22]

Much of the published research evaluating the performance of serologic tests for the detection of antibodies to *B burgdorferi*, including those articles

referenced by current criteria for serologic interpretation, often does not report sex-specific analyses or sample demographics.[23–26] A recent article compared the proportion of seropositive males and females in a sample of culture-confirmed, acute patients from New York and concluded that seropositivity was unaffected by the patients' sex.[27] The previously mentioned prospective clinical study from Sweden found differences in seropositivity following infection.[21] Results from a community survey of serologic data conducted in 1993 found differences in seropositivity by age and sex, particularly by ELISA; the authors concluded that false-positive results were more common among women.[28] In the latter study, patients were not characterized clinically, nor were serologic tests interpreted according to more recently published criteria.

The small body of research conducted to date on sex differences in early Lyme disease does not conclusively address whether differences observed in other infectious diseases are relevant to this setting, leaving an important gap in our understanding of clinical response to this infection and the sensitivity of currently accepted criteria for diagnosis and treatment.

METHODS
Patient Sample

This article presents data from a retrospective chart review of consecutive, adult patients presenting between August 2002 and August 2007 to a general internist with infectious disease training (J.N.A.) for possible Lyme disease. The practice is located in Baltimore County, Maryland, a region characterized as high risk for Lyme disease.

Patients had been either physician or self-referred for consultation, and had presented with symptoms of variable duration and degree of evidence for past or current infection with *B burgdorferi*. For all patients, a complete, verbal medical history had been obtained, and all relevant medical records had been reviewed for clinical findings from the time of illness onset. All patients were evaluated by one physician (J.N.A.); however, medical records were abstracted if patients had been evaluated previously by another physician during the course of their illness. Acutely ill patients had been further evaluated by physical exam for EM or other objective findings consistent with early Lyme disease. All patients had been extensively evaluated if a diagnostic EM was not found at the time of consultation, or if the medical history, physical exam, or laboratory findings were suggestive of a possible alternative dermatologic, neurologic, or cardiac diagnosis. All clinical findings and results of serologic testing were then abstracted from patient medical records and entered into an electronic database through an abstraction form. Additional relevant variables abstracted through medical record review of patient self-report relating to illness onset were also entered into the database.

The analyses presented herein include only those patients from this community-based sample meeting the CDC surveillance case definition for confirmed early Lyme disease prior to or during our evaluation. This group is defined by a history of physician-documented EM or of meningitis, VII nerve palsy, or carditis, and a concurrent positive serologic test for Lyme disease.[29,30] Final determination of confirmed early Lyme disease status was reviewed by a clinician (J.N.A.) to ensure accuracy. We restricted the sample in this way to ensure including only those with the strongest degree of evidence available to clinicians for true exposure to infection with *B burgdorferi*.

This chart review was approved by the institutional review board of the Johns Hopkins University School of Medicine.

Interpretation of Serologic Results

All serologic tests for Lyme disease performed within 3 months of illness onset were abstracted from patient medical records and included if performed by commercial laboratories implementing 2-tier testing according to CDC criteria. These criteria were also used to interpret results and determine seropositivity of patients in this sample: an IgM Western blot with at least 2 of the 3 possible bands present was considered positive, whereas an IgG Western blot with at least 5 of the 10 bands present was considered positive.[8] As recommended by the CDC and the Infectious Diseases Society of America, all serologic tests performed within

4 weeks of illness onset were considered positive by 2-tier testing if the ELISA and either the IgM or the IgG were positive. As a positive IgM alone is not considered reliable after 4 weeks of infection, all tests performed after this point were considered confirmatory only if the IgG was also positive.[8,29,30] Serologic data were categorized dichotomously as positive/negative and also analyzed as continuous measures to look for differences in quantitative variation. This latter approach has been used historically to determine cut-off points for positivity,[24] and more recently, has been shown to increase sensitivity of the Lyme Western blot through the calculation of high- and low-risk thresholds.[31]

Statistical Methods

Differences in clinical characteristics by sex were assessed using Wilcoxon rank sum tests for continuous variables and Fisher exact tests for categorical variables. Enhanced box plots were used to display distributions of ELISA values and the number of IgG bands by sex.[32] Quantile regression was used to determine whether specific percentiles of ELISA values were different between men and women.[33] Separate Wilcoxon rank sum tests were used to test for differences between men and women in the number of IgM and IgG bands present. Results with a 2-sided P value <0.05 were considered statistically significant.

RESULTS
Acute Clinical Characteristics

A total of 125 patients had presented for evaluation and met the CDC surveillance case definition for a current or past episode of confirmed early Lyme disease. The physician-documented, clinical characteristics of this acute episode are shown in **Table I**. Demographically, this sample was 50% female and the median age at illness onset was 48 years. The restriction of our sample to patients with confirmed early Lyme disease is evident in the elevated proportion of patients with EM (91%) or another objective finding (18%). In addition, 54% of patients presented with a flu-like illness at onset. None of the clinical characteristics measured, including both initial physician and self-misdiagnosis, were significantly different between men and women. The proportion of men and women with multiple EM was 27% and 16%, respectively (P = NS), and the initial self-misdiagnosis rates for men and women were 10% and 18% (P = NS).

Table I. Chart review of acute characteristics overall and by sex among 125 adult patients with a history of confirmed early Lyme disease. Values are number (%), unless otherwise indicated.

Characteristic	Overall (N = 125)	Men (n = 63)	Women (n = 62)	P*
Age at illness onset, y[†]	48 (37–57)	48 (39–62)	48 (37–54)	0.36
Physician-documented EM[‡]	114 (91)	56 (89)	58 (94)	0.53
Multiple physician–documented EM[‡]	27 (22)	17 (27)	10 (16)	0.19
Other objective finding at onset[‡§]	22 (18)	12 (19)	10 (16)	0.82
Flu-like illness at onset[‡]	68 (54)	32 (51)	36 (58)	0.47
Initial physician misdiagnosis	25 (20)	14 (22)	11 (18)	0.66
Initial patient self-misdiagnosis	17 (14)	6 (10)	11 (18)	0.20
≥1 Serology performed	62 (50)	28 (44)	34 (55)	0.29
Patients with seropositive test result[‖]	25/62 (40)	14/28 (50)	11/34 (32)	0.20

EM = erythema migrans.
*Determined by Wilcoxon rank sum test for age at illness onset and by Fisher exact test for all other characteristics.
[†]Median (interquartile range).
[‡]Categories are not mutually exclusive; some patients presented with >1 finding.
[§]Includes meningitis, VII nerve palsy, and carditis.
[‖]Patients with ≥1 serology performed.

Serologic Reactivity

Sixty-two (50%; 28 men, 34 women) patients in this sample had ≥1 serologic test performed as part of their clinical evaluation, while the remaining 63 (50%; 35 men, 28 women) without an acute serologic test had been clinically diagnosed with EM and the diagnosing physician had not sought serologic confirmation. Again, there were no statistically significant differences by sex in any of the clinical variables from **Table I** among this subset of 62 patients with a serologic test (data not shown).

Among the 62 patients with ≥1 serologic test performed, 25 (40%) had a positive, 2-tier result within 3 months of illness onset (**Table I**). Fourteen (50%) of the 28 men tested for Lyme disease had at least 1 seropositive test, while 11 (32%) of the 34 women did (P = NS). Nineteen (31%) of the 62 patients tested had >1 serology drawn during the 3-month time frame; in all cases, this occurred if an initial test returned negative and a second was ordered, as recommended by current testing guidelines. The proportion of patients with >1 test was not significantly different by sex (25% of men vs 35% of women). For these patients, only the later test was included in the analyses to capture the highest proportion of seropositive patients and to minimize the effect of the early seronegative window in both sexes.

A summary of the proportion of men and women with positive ELISA, IgM, or IgG results is shown in **Table II**. Seventy-one percent of men and 62% of women were ELISA positive, the proportion of IgM-positive patients was almost identical by sex, and 40% of men and 19% of women were IgG positive (all, P = NS). There were no significant differences by sex in either illness duration prior to serology, proportion of patients initiating antibiotics prior to confirmatory serology, or proportion of patients with disseminated lesions. These 3 variables have been shown to be associated with serostatus in early Lyme disease.[26,27,34]

We examined the distribution of ELISA values and the distribution of the number of IgM and IgG bands by sex. **Figure 1** shows enhanced box plots of the distribution of ELISA values of the 20 men and 21 women with positive or equivocal ELISA. The median ELISA values among men were significantly higher (3.4 vs 2.0; P = 0.03) and the 25th, 75th, and 90th percentiles of ELISA values among men were significantly higher than the corresponding percentiles among women (all, P < 0.05). There were no significant differences by sex in the number of IgM bands present (0% of men and 10% [n = 2] of women had 0 bands, 15% [3] of men and 5% [1] of women had 1 band, 35% [7] of men and 52% [11] of women had 2 bands, and 50% [10] of men and 33%

Table II. Serologic tests performed within 3 months of illness onset by sex among a sample of 62 patients with confirmed early Lyme disease. Values are number (%), unless otherwise indicated.

| Variable	Men (n = 28)	Women (n = 34)	P*
ELISA positive or equivocal	20/28 (71)	21/34 (62)	0.59
IgM positive among ELISA positive or equivocal†	17/20 (85)	18/21 (86)	1.00
IgG positive among ELISA positive or equivocal†	8/20 (40)	4/21 (19)	0.18
Time from illness onset to serology, d‡	22 (10–31)	25 (8–56)	0.23
Antibiotic exposure prior to serology§	14/28 (50)	19/33 (58)	0.61
Multiple physician-documented EM	8/28 (29)	6/34 (18)	0.37

Ig = immunoglobulin; EM = erythema migrans.

*Determined by Wilcoxon rank sum test for time from illness onset to serology and by Fisher exact test for all other characteristics.

†Two or more of 3 IgM bands required for IgM positivity; 5 or more of 10 IgG bands required for IgG positivity.

‡Median (interquartile range). Missing data on time to serology for 1 woman.

§Missing data on antibiotic exposure for 1 woman.
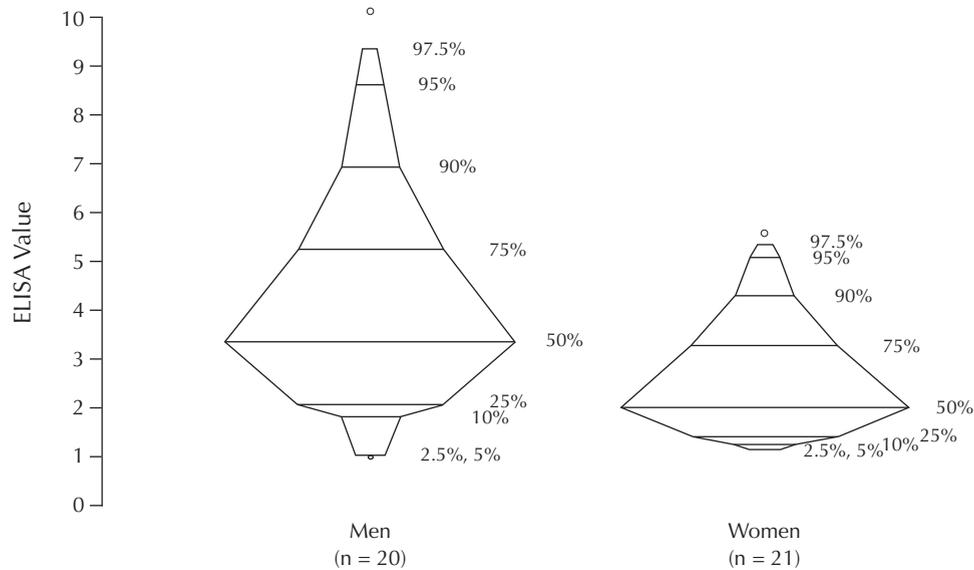
Figure 1. Distribution of ELISA results among men and women with a positive or equivocal value (>0.90) by sex. Median values were 3.4 for men and 2.0 for women (*P* = 0.03).

[7] of women had 3 bands). In contrast, **Figure 2** shows that compared with women, men had a significantly greater number of IgG bands present (10% [2] of men and 43% [9] of women had 0–1 bands, 35% [7] of men and 33% [7] of women had 2–3 bands [median number of bands for women = 2], 30% [6] of men and 14% [3] of women had 4–5 bands [median number of bands for men = 4], 10% [2] of men and 5% [1] of women had 6–7 bands, and 15% [3] of men and 5% [1] of women had 8–9 bands; *P* = 0.03).

DISCUSSION

This study examined clinical and serologic differences by sex among a community case series of patients with a current or past episode of confirmed early Lyme disease. This represents one of a small number of studies to examine this hypothesis, particularly among patients with representative medical histories including prior misdiagnosis, antibiotic exposure, and variable illness durations. There was no significant difference in clinical presentation among male and female patients in our sample; however, there was a high rate of initial physician and patient misdiagnosis among both sexes. Patients often incorrectly attributed EM to nonspecific insect or spider bites and failed to recognize its significance. In conjunction with an initial physician misdiagnosis rate of 20%, these findings suggest a continued need for education and an awareness that both patient and physician misdiagnosis remain problematic in community settings. Furthermore, our sample included only patients with more classic presentations of early Lyme disease, thus overestimating the proportion of patients presenting with EM. In the 20% to 30% of patients with viral-like syndromes but no EM, or among those patients with atypical or unobserved EM, misdiagnosis is likely to occur more frequently. Serologic antibody testing remains the recommended tool for confirming a diagnosis of Lyme disease among these patients.[29,30]

In our sample, 40% of tested patients had a positive serologic result on commercial 2-tier testing within 3 months of illness onset. Even though a higher proportion of women were later retested for antibody development during this interval, only a third tested positive at any point over the 3 months, compared with half of the men. The ELISA and IgG components appeared to contribute more to this discrepancy, as IgM seropositivity was nearly identical among men and women. These findings may be important, as the ELISA
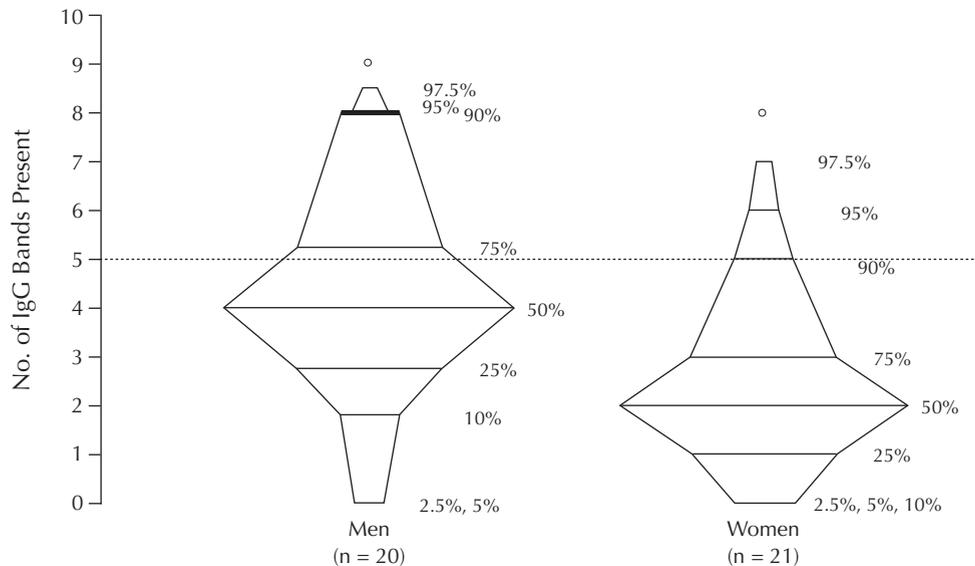
Figure 2. Distribution of number of immunoglobulin G (IgG) bands present among men and women with a positive or equivocal ELISA result (>0.90) by sex. Median number of bands were 4 for men and 2 for women ($P = 0.03$).

serves as a first-tier screening tool to determine appropriateness of further IgM/IgG testing, whereas IgG is used as a marker for late disease. These findings may also offer an explanation for higher incidence rates among males,[1] particularly after 1995 and concurrent with changes to serologic criteria requiring IgG positivity in confirming disease of >4 weeks' duration.[8]

Further studies are needed to replicate our findings. If sex-based differences are found to be present, they would have implications both for public health surveillance and clinical diagnosis of Lyme disease. Specifically, robustness of IgG antibody development would be of particular importance to late-disease classification. Diagnostic criteria for late Lyme disease include an objective clinical manifestation, often arthritis, and a positive IgG serology after 4 weeks from illness onset.[29,30] In series of patients with late Lyme arthritis, men appear to be overrepresented (by a ratio of 2:1) to a greater extent than the 53% majority of all early and late Lyme cases reported to the CDC would suggest.[35–37] It is unknown whether men are more likely to present with late-disease manifestations meeting these criteria; however, our findings may suggest an alternative explanation—that variation in IgG seroconversion by sex may render criteria

for late Lyme disease more restrictive for women. It is also unknown whether serologic testing later in the illness would show a delayed response among women. However, in one non–sex-differentiated study, antibody levels began to fall among antibiotic-treated patients as quickly as 30 days after infection.[26]

Outside the framework of objective late Lyme disease, an additional subset of ill-defined, controversial patients is often diagnosed with "chronic Lyme disease." Patients with chronic Lyme disease present with varying degrees of past or present seropositivity and a clinical phenotype of mainly subjective symptoms.[38] A recent review article identified this subset of patients as predominantly (69%) female.[39] It has also been suggested that chronic Lyme may represent misdiagnosed fibromyalgia or chronic fatigue syndrome, also female-dominated syndromes of unknown etiology. The association between Lyme disease and fibromyalgia, particularly among women, was suggested by results from a previous case series.[40] Future studies of sex-based differences in the context of Lyme disease will thus need to address the immunology of antibody development, the epidemiology of clinical disease manifestations, and the implications of current sex-based diagnostic discrepancies in late and chronic disease.

The effect of several potential confounders, although not univariately associated with sex in our data, should be considered as well. Longer illness duration prior to serology, as well as the presence of disseminated lesions, has been found to be associated with seropositivity.[27] There is also evidence that exposure to antibiotics may blunt antibody development,[35] and that this effect may be more pronounced for IgG rather than IgM.[26] More than half of the patients in our community sample had been prescribed an antibiotic prior to confirmatory serologies, including those mistakenly prescribed antibiotics ineffective for treatment of early Lyme disease.

High rates of prior antibiotic exposure may partially explain the discrepancy between our findings and those of Wormser et al,[27] as all patients in the latter study were antibiotic naive. The modifying effect of antibiotic exposure on the robustness of IgG antibody response for males and females may thus be significant and merits studies with larger sample sizes. The longer illness duration of patients in our sample may also have contributed to this discrepancy. All patients in the Wormser study had an illness duration of <30 days, and therefore it can be inferred that IgM reactivity strongly contributed to the 2-tier serologic results presented. In this case, any IgG-specific effect would be diluted or overlooked. Finally, we are unaware of any studies that have controlled for the known clinical confounders listed in **Table II** for the relationship between sex and serologic reactivity in Lyme disease.

There are several limitations to this study. First, we were unable to perform cultures or polymerase chain reaction tests on patients and therefore could not definitively confirm the documented episodes of early Lyme disease. However, physicians in community practice likewise do not have access to these tools and must rely, as we did, on clinical expertise when evaluating patients and past medical records. Second, the retrospective nature of the data relied on results from several commercial laboratories and physician offices. Excluding tests from laboratories not adhering to CDC criteria may have controlled for some, but not all, interlaboratory variability. Serologic test results included in this analysis may have come from different commercial laboratories using different antigen preparations. These factors, along with an inability to screen for patients with a prior history of Lyme disease, could introduce variability into the serologic data included in our analyses. Thus, we would caution against overgeneralization of pooled ELISA, IgM, and IgG results beyond our population of interest: patients seen in community practice. However, we feel that the sex-based comparisons drawn from our data may still be valid, as we have no a priori reason to believe that any of the factors mentioned would be distributed differentially among males and females. Third, by nature of the study design, patients in our sample presented with high rates of EM and other objective findings. Other, less easily recognizable clinical presentations may offer additional insight into the potential for biologically or sociologically driven sex-based differences in early Lyme disease. Finally, because a relatively small number of men and women were used in many of the comparisons that we made, additional studies with larger sample sizes are needed to determine whether our findings are replicable.

In many settings, the accurate identification of infectious disease relies on serologic evidence of exposure. Despite limited sensitivity in early diagnosis and frequent misuse by physicians, serologic testing in early Lyme disease remains central to diagnosis and case reporting. It is known that seroconversion does not occur in a minority of patients with early Lyme disease and may be further blunted by exposure to antibiotics prior to serologic confirmation. In cases of Lyme disease of >4 weeks' duration, emphasis is placed on IgG seroconversion. If the IgG response is indeed less robust among women, our findings would thus endorse the suggestion that the interval during which IgM positivity can be used to support the diagnosis of early Lyme disease should be extended beyond 4 weeks.[41]

CONCLUSIONS

This retrospective study examined sex differences in the clinical and serologic presentation of confirmed early Lyme disease. We did not find signifi-

cant differences by sex in the clinical variables we included in the analysis. However, our data did suggest the potential for sex-based differences that might place women at an additional disadvantage for ELISA and IgG seroconversion during an episode of early Lyme disease. Prospective studies with larger, population-based samples are needed to determine whether the sex differences we observed in serologic response to confirmed early Lyme disease are replicable and if so, whether they are clinically relevant or immunologically significant.

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