Low Seroprevalence of Lyme Disease Among Multiple Sclerosis Patients in New Brunswick

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ABSTRACT: The signs and symptoms of Lyme neuroborreliosis can overlap with non-infectious degenerative diseases such as multiple sclerosis (MS). In this study, we assessed a cohort of MS patients in Atlantic Canada for serological evidence of Lyme disease (LD). No positive serology was identified using the recommended two-tiered algorithm.

RÉSUMÉ : Faible séroprévalence de la maladie de Lyme parmi des patients du Nouveau-Brunswick atteints de sclérose en plaques. Les signes et les symptômes neurologiques associés à la maladie de Lyme (neuroborréliose) peuvent recouper ceux de maladies dégénératives non-infectieuses comme la sclérose en plaques (SP). Dans cette étude, nous avons fait l'évaluation d'une cohorte de patients du Canada atlantique atteints de SP afin d'obtenir des preuves sérologiques de la maladie de Lyme. De façon générale, aucune sérologie positive n'a été identifiée au moyen d'un algorithme à deux niveaux (*two-tiered algorithm*) recommandé.

Keywords: Lyme disease, *Borrelia burgdorferi*, Multiple sclerosis, Seroprevalence

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Lyme disease (LD) is a zoonotic infection caused by bacteria belonging to the *Borrelia burgdorferi sensu lato* complex (Bb), which is transmitted to humans by infected *Ixodes* ticks. If the infection is not treated, the bacteria can disseminate leading to other manifestations such as arthritis and carditis, as well as neurologic disease called Lyme neuroborreliosis (LNB). The signs and symptoms of LNB are variable and can affect both the central and peripheral nervous systems.[1] Regional differences in the presentation of LNB have been noted and are likely due to variations in *B. burgdorferi* genospecies. LNB is more commonly identified in Europe and associated with painful radiculitis and chronic progressive spastic paraparesis. In North America, cranial neuropathy or aseptic meningitis are the main manifestations of LNB cases.[1] The symptoms of LNB can overlap with other non-infectious degenerative diseases such as multiple sclerosis (MS), Parkinson's disease, and amyotrophic lateral sclerosis (ALS). Furthermore, there are data to suggest that patients with LNB may present with MRI findings in the CNS;[2,3] however, these radiologic findings are non-specific, and similar lesions can be found in other demyelinating diseases and also in normal controls.[2] Given that the Atlantic provinces have the highest rates of MS in Canada, and have increasing rates of Lyme disease,[4,5] in this study, we assessed a cohort of patients known to have MS and living in the Atlantic Canadian province of New Brunswick, for serological evidence of Lyme disease.

Participants were recruited from the MS Clinic in Saint John, New Brunswick, between June 16 and July 22, 2014, at the time of scheduled clinic appointments. To avoid selection bias, all clinical patients were approached to enroll in the study until the target sample size was met. All participants had been diagnosed with MS by a neurologist, based on neurologic findings, new lesions on MRI demonstrated over time, and in some cases, positive cerebrospinal fluid (CSF) banding, in accordance with the McDonald criteria or the older Barkhoff's criteria.[6] Once informed consent was obtained, patient sera, demographic information, MS type and history, tick exposure history, and employment information were collected. The presence of Bb antibodies in each participant's sera was determined using the two-tiered algorithm in accordance with the CDC guidelines. Briefly, sera were tested with the C6 *B. burgdorferi* ELISA (Immunetics, Inc., Boston, Massachusetts, USA) as per the manufacturer's recommendations. Positive (antibody index $\geq$ 1.10) and equivocal (antibody index 0.91–1.09) specimens subsequently underwent IgG Western blot (WB) testing (*B. burgdorferi* US (IgG), Euroimmun, Luebeck, Germany). Samples were considered positive based on the CDC interpretive criteria which require 5 of 10 bands of sufficient intensity were present on the IgG Western blots.[7] The seroprevalence of Bb antibodies in the MS patient cohort was compared to a group of 74 healthy individuals with no neurologic conditions recruited from within New Brunswick. The controls were recruited

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Table 1: Description of the Western blot banding patterns associated with positive and equivocal C6 EIA specimens with testing at the clinical and research laboratories

Patient	Results					
	C6 EIA reactivity		Bands present on IgG WB		Standard two-tiered testing using CDC criteria	
	NSHA	MAU	NML	MAU	NSHA/NML	MAU
MS-22	Positive	Negative	41	41, 60[a]	Negative	Negative
MS-29	Positive	Negative	None	58	Negative	Negative
MS-52	Positive	Positive	41	30, 41, 50[a], 57[a]	Negative	Negative
MS-55	Positive	Positive	66	None	Negative	Negative
MS-63	Equivocal	Negative	41	None	Negative	Negative
MS-65	Positive	Negative	41	41, 57[a], 58, 60[a]	Negative	Negative
MS-66	Positive	Positive	41, 66	39, 41, 57[a], 60[a]	Negative	Negative
Control-17	Positive	Positive	Negative	23, 57[a]	Negative	Negative
Control-125	Negative	Positive	23/25, 41, 83/93	34[a], 38[a], 39, 41, 45, 57[a], 58, 60[a], 66[a], 83[a]	Negative	Positive
Control-126	Negative	Positive	41	28, 41, 58, 60*	Negative	Negative

EIA – enzyme immunoassay; NSHA – Nova Scotia Health Authority clinical laboratory; MAU – Mount Allison University research laboratory; NML – National Microbiology Laboratory.
*Bands are not considered in the CDC interpretive criteria.

through a Mount Allison University (MAU) study and were geographically matched, though not matched for age or gender.

In this study, the first-tier EIA testing using the C6 EIA was performed in two separate labs: the clinical laboratory responsible for LD testing in Nova Scotia and New Brunswick (QEII Microbiology Laboratory, Nova Scotia Health Authority (NSHA), Halifax, Nova Scotia), and a research laboratory at MAU in Sackville, New Brunswick. Positive EIAs identified at the NSHA underwent confirmatory WB testing at the National Microbiology Laboratory in Winnipeg, Manitoba. The MAU research lab also performed WB testing on study specimens (MarDX *B. burgdorferi* IgG Marblot Western Blot). Continuous data were analyzed using the *t* test.

Ninety patients with MS participated in the study. Most were female (66/90) with a mean age of 51 years (+/− 12 yrs.) and 30 (33%) were receiving immunomodulatory therapy consisting of natalizumab, fingolimod, dimethyl fumarate, or teriflunomide. All participants were from southern New Brunswick which has the highest number of LD cases in the province.[5] Twenty-one of 90 (23.3%) study participants described prior tick exposure. Only 7/90 (7.8%) MS patients had a reactive or equivocal C6 EIA. Overall, there was no difference between the average C6 EIA index values obtained at the two laboratories (0.46 vs 0.42 for NSHA and MAU, respectively; p=.09). In addition, at both labs, the average C6 EIA index was significantly lower when comparing patients that were on immunosuppression to those that were not (0.26 vs 0.56, $p < 0.001$, and 0.33 vs 0.47, p=.045 for NSHA and MAU laboratories, respectively). All confirmatory IgG WBs were negative based on CDC criteria. Of the seven reactive or equivocal C6 EIA specimens, five had a single reactive band, one had two bands and one did not have any reactive bands on the IgG WB (Table 1).

In the healthy control population, only 1/74 had a positive C6 EIA at both CDHA and MAU labs. The IgG WB result was negative (only 2/10 bands were present) for this control specimen at both labs. Testing of controls at the MAU research lab also noted two other specimens that were positive by EIA. These two specimens were negative at the clinical laboratory at NSHA, however, and also negative by IgG WB testing at the NML (3/10 bands and 1/10 bands).

In this study, none of the MS patients or healthy controls had positive LD serology using the recommended two-tiered algorithm.[7] Although there were reactive or equivocal C6 EIAs in the MS cohort, the use of the C6 EIA alone is not recommended because it has a lower specificity than the two-tiered algorithm leading to falsely reactive results. When these specimens were tested using a commercially available IgG WB, 6/7 had one or two bands on the IgG WB. The most common was p41 which is known to have poor specificity as antibodies to other bacteria can cross react with this protein.[8]

There have been a number of studies in the US and Europe that have looked at the seroprevalence of Bb antibodies in MS patients. A study in New York showed that only 1/89 patients from Long Island with a definite diagnosis of MS had a positive Bb EIA (using a whole-cell sonicate) which was felt to be secondary to prior exposure to Lyme disease in an endemic region.[9] Similarly, a study in Austria found that there was no difference in the seroprevalence in 106 MS patients compared to 13 matched controls.[10] There are other data, however, that have found higher seroprevalence in MS patients compared to controls or other neurologic disease.[11,12] However, these studies did not use the two-tiered algorithm. One study used IFA[12] which is not commonly used for Lyme serology and the other studies used only EIA based on the whole-cell sonicate of Bb.[9–11] None of the

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studies tested their reactive samples with WB. These are significant limitations as the whole-cell Bb EIA is known to have poor specificity ranging from 86.3% to 96.1%.[13]

In another study from a highly endemic LD area in Norway, 12/179 (7%) MS patients had Bb antibodies detected in serum which was much lower than 18% identified in blood donors. Of these MS patients with positive serology in blood, none had antibodies in their CSF.[14] The documentation of intrathecal antibody production using an antibody index (AI) has a sensitivity approaching 100% when patients have had symptoms longer than 8 weeks.[15] The authors do not describe whether the serologic results in blood were from EIA alone or using the two-tiered algorithm. However, even if the seroprevalence was determined using the appropriate two-tiered testing, given the lack of intrathecal antibodies, and, therefore, a negative AI, it is unlikely that the MS patients with antibodies in their serum had LNB, rather the positive serology likely reflects previous exposure to Bb while living in a highly endemic LD area.

One-third of patients in this study were on immunomodulatory agents including natalizumab, fingolimod, dimethyl fumarate, or teriflunomide. While well below the assay cutoff for positivity, the lower C6 index values in these patients raise the possibility that treatment with these agents could have affected the ability of the MS patients to generate an antibody response to Bb. In MS patients with history and symptoms consistent with LD, performing serologic testing prior to initiation of immunomodulatory therapy should be considered.

Although our sample size was small, our study does not suggest that LNB is being missed in MS patients in the Atlantic Canadian province of New Brunswick.

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Statement of authorship

GM was involved in developing the study concept and design. PC coordinated the study, including the recruitment and enrollment of study participants and acquisition of sera for analysis. LRL oversaw confirmatory Western blot testing and assisted with data analysis and manuscript preparation. TFH provided EIA testing of study and control group sera, analyzed data, and assisted with writing of the manuscript. DW assisted in study planning and logistics, data analysis, and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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