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Original article

Epidemiology of Lyme disease among US Veterans in Long Island, New York

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ABSTRACT

In North America, Lyme disease **(LD)** is caused **predominantly** by the spirochete *Borrelia burgdorferi* **sensu stricto, and is** transmitted by blacklegged ticks. Long Island, New York, is highly endemic for the disease. The C6 peptide (C6P) is currently used **as a screening test for LD** in our institution**. Our objective was to examine how screening with C6P concorded with diagnosis of LD** at the Veterans Affairs Medical Center, Northport, Long Island. A retrospective chart-review of 2558 C6P tests was performed during the period of 1/1/2010 to 12/31/2016. Patients were categorized **by Lyme Disease (LD) or no LD groups.** LD group was defined as having an erythema migrans (EM) rash, or ≥ 2 IgM bands or ≥ 5 IgG bands on immunoblot. Out of the 409 patients with positive or equivocal C6P, **181 patients with LD** were based on presence of EM, or **W**estern blot IgM and IgG test results; 228 **did not have LD**. The positive predictive value **of C6P** was 44.5%. EM was the most common presentation. In the LD group, history of tick bite (P: 0.0001), headache (P: 0.0036), joint swelling (P: 0.0086) and myalgias (P: 0.0005) were more likely to be present. Zip code mapping of our cases mirrored those previously reported in the Suffolk County Department of Health. In our review we encountered a significant number of false positive C6 assays. False positive C6P tests were ordered by primary care physicians (PCP) (37%) followed by neurologists (33%). A history of tick exposure and clinical findings of early Lyme disease such as headaches or joint aches were more likely to denote a true positive C6 peptide test. Rigorous education of physicians about Lyme disease and pitfalls of our available diagnostic tests are needed for their proper utilization.

1. Introduction

Lyme disease (LD) is the most common vector-borne disease in the United States (Hu, 2016). It is transmitted by an infected blacklegged tick bite, *Ixodes scapularis,* and caused by a spirochete, *Borrelia burgdorferi* **sensu stricto**. Blacklegged ticks can be found in many parts of the country. Long Island, New York, is considered highly endemic for both blacklegged ticks as well as LD (Hu, 2016). LD is **seen predominantly** in North America and Europe. The skin lesion, erythema migrans (EM), is the only specific marker for early LD and **is** present in approximately 80% of acutely diagnosed individuals (Steere et al., 1998). Other clinical manifestations for LD are nonspecific. The Center for Disease Control (CDC) in 1995 recommended a two tier system for diagnosis of LD consisting of screening immunoassay followed by confirmatory Western Blot (WB). The first tier test is an enzyme linked immunosorbent assay (ELISA) or immunofluorescence assay (IFA) that measures total antibody response to spirochetal antigens. The WB acts as the second tier test; it is an IgM and IgG immunoblot against *B. burgdorferi* whole cell sonicate (Wormser et al., 2013). This two tier testing system has low sensitivity in early infection. The sensitivity of current IgM and IgG LD assays during early infection seldom exceeds 50% (Nowakowski et al., 2001**). Recently, the C6 peptide, a new testing modality** has gained popularity in **LD diagnosis** (Moore et al., 2016). The C6 peptide (C6P) is an antigen on the segment of VIsE (Variable major protein-like sequence) an outer surface protein on *Borrelia* species. It is a highly immunogenic conserved region. C6P has increased sensitivity in early LD compared to the two-tier system (Wormser et al., 2013). It has comparable sensitivity in later stages of

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the disease but has decreased specificity (Wormser et al., 2013). However, its specificity can be increased by supplemental immunoblot testing (Lipsett et al., 2016). In our institution, C6P is used as a sensitive screening test for LD, rather than **other commercially available Lyme ELISAs**. Positive or equivocal C6P result is followed by **reflex** immunoblot confirmation testing. In **this** study, we retrospectively reviewed and **examined how screening with C6P concorded with clinical illness leading to an epidemiological understanding of LD among our patients.**

2. Materials and methods

The Northport Veterans Affairs Medical Center is located in Suffolk County, Long Island, New York. The electronic medical records were reviewed from 1/1/2000 to 12/31/2016; during this period, 2558 Lyme C6P tests were performed. The C6P is an enzyme linked immunosorbent assay (Immunetics® C6 Lyme ELISA™ Kit, Boston Massachusetts). C6P **test value** results are defined as: ≤ 0.90 negative; 0.91–1.09 equivocal; ≥ 1.10 positive. Positive or equivocal C6P tests underwent reflex testing with WB: MarDx® *B. burgdorferi* (IgM)/(IgG), Carlsbad California. A positive IgM WB is defined as presence of any two or all of the following bands: 23, 39, **41 kDa.** A positive IgG WB is defined as presence of any five, or more, of the following bands: 18, 23, 28, 30, 39, 41, 45, 58, 66, **93 kDa**. Positive or equivocal C6P results were observed in 409 unique subjects. If a patient had multiple C6P tests, **the earliest test result was included in data analysis.** From the electronic medical records the following data were obtained: age of patient at time of test, demographic information, zip code of residence, ordering service, history of tick bite, presenting symptoms if any, and recording of positive bands of the WB. Treatment and antibiotic choices were recorded as well as outcomes. Dichotomous and categorical variables were compared using $\chi 2$ test or Fisher's exact test. Frequencies were obtained by using Microsoft Excel®. The study was approved by the Institutional Review Board.

Lyme Disease **Case** Definitions: Early **localized** LD is defined as classic EM presentation. Immunoblot test is expected to be negative; however, C6P can already be positive. A positive C6P and concurrent positive WB would define a case of LD in the presence of appropriate disease-associated clinical syndrome. The latter syndrome covers early disseminated LD with multifocal EM, carditis, meningitis, cranial neuritis (seventh nerve palsy most common) and radiculoneuritis; and late LD with arthritis. If cerebrospinal fluid or synovial fluids were sent for *B. burgdorferi* PCR testing, the results were reviewed but positive results were not required to establish the diagnosis. A patient with positive immunoblot test but with no active symptoms and no history of LD is considered to be exposed to *B. burgdorferi.* A false positive C6P is defined in a patient with negative immunoblot and no clinical suggestion for any form of LD or if clinical presentation was explained by different medical diagnosis. Positive predictive value for the C6P was calculated as the proportion of positive C6P results that deemed to be true positive.

Lyme Disease Surveillance Suffolk County Department of Health: The Suffolk County Tick and Vector-Borne Diseases Task Force (TVBDTF) presents and overviews important topics and recommendations related to vector-borne diseases in Suffolk County, primarily tick-borne and mosquito-borne pathogens and associated diseases. The TVBDTF was created by the Suffolk County Legislature as Resolution #689-2011 to "study the spread of tick and vector-borne diseases, and to develop a comprehensive needs assessment for the County's approach to this public health and safety issue."

The New York State Department of Health (2013) has developed a system to estimate the prevalence of LD per each County based on a 20% random sample of positive laboratory reports (Lukacik et al., 2018). This methodology produces accurate estimated LD case counts and has been used successfully by the TVBDTF to demonstrate rising incidence of all tick-borne associated diseases in the last decade.

Table 1
Clinical Presentations in 181 patients with Lyme Disease.

Clinical Presentations in 181 patients with Lyme Disease
Asymptomatic 9
History of tick exposure 4
History of Lyme 1
Peripheral neuropathy work 14
Erythema Migrans 32
Seventh Nerve Palsy 5
Palpitations 2
Headache/Neck pain 27
Joint pain 58
Knee 19
Hip 5
Wrist 5
Elbow 1
Polyarthralgias 28
Myalgias/Fatigue 34

3. Results

Four hundred and nine patients had positive or equivocal C6P and concurrent supplemental immunoblot tests. The majority were men. There were 181 patients with LD and 228 without LD. The 181 patients had both C6P and WB positive. The positive predictive value **of C6P** was 44.5%. Tables 1 and 2 show the clinical presentations or reasons for **ordering** the test in each group. Descriptive statistics for the study sample are shown in Table 3. EM was the most common presentation. In the LD group the following were more likely to be present: history of tick bite (P: 0.0001), headache (P: 0.0036), joint swelling (P: 0.0086) and myalgias (P: 0.0005). In five LD patients the seventh-cranial-nerve palsy was observed. Three patients with headache attributed to LD had evaluation with lumbar puncture and the cerebrospinal fluid PCRs for *B. burgdorferi* **sensu stricto** were negative.

Table 4 shows the antibiotic treatment choices and outcomes. Thirty-two patients presented with EM had positive C6P; 6 of them had positive immunoblot while 26 had negative immunoblot. Fig. 1 shows frequencies of positive IgM and IgG bands **between the two groups**. The IgG band, 41 kD, was the most frequently isolated band in **the group without LD** (76.3%). The most frequent bands in the **LD group** were 39 kD (70.7%), 18 kD (67.4%), 58 kD (61.3%), and 66 kD (59.6%).

Of patients with an initial positive C6P result who subsequently had repeated C6P tests during the study period, 39 had multiple tests (two to four times) that remained positive, and in some cases the test continued to be positive for up to 5 years. Twenty-one patients' repeat C6P tests resulted equivocal.

Fig. 2 shows the map of Long Island **with the cases of LD per zip code diagnosed in our Veterans**. Fig. 3 shows **overall cases** of Lyme activity as reported by the Suffolk County Department of Health. **It is interesting to point out the similarity of LD activity, especially the high-density regions, seen in both maps that of our institution's and the Suffolk County.**

As the tick vector can also transmit *Babesia microti* and *Anaplasma phagocytophilum* we searched for possible co-infections in our patients. Seven patients had babesiosis with positive smear and parasitemia ranging from 0.1% to 3%; two of them had negative Lyme immunoblot. Another patient with babesiosis had history of splenectomy and required exchange transfusion. Two LD patients had anaplasma IgM antibody positive and one with positive *A. phagocytophilum* PCR.

4. Discussion

Our Veterans live in a highly endemic area for LD and blacklegged hard ticks. Co-infections with *B. microti* and *A. phagocytophilum*, both transmitted through the bite of blacklegged ticks, were observed.

Table 2
Clinical Presentations in 228 without Lyme Disease.

Clinical Presentations in 228 Patients without Lyme Disease
Asymptomatic 55
History of Tick Bite 9
Patient's dog with Lyme 1
Peripheral neuropathy work up 41
HIV- 1
B12 deficiency 3
Diabetic 1
Carpal Tunnel syndrome (confirmed by EMG) 2
Polyneuropathy (confirmed by EMG) 5
Ulnar neuropathy (confirmed by EMG) 1
Eye Complaints 8
Choroiditis 1
Blurry vision 1
Uveitis 2
Iritis 2
Glaucoma 1
Diplopia 1
Tinnitus/Hearing Loss 6
Other Tick Borne diseases 2
Babesiosis 2
Myalgias 17
Fibromyalgia 4
Headache 19
Memory difficulties 8
Cellulitis 7
Chronic Fatigue 4
Syncope 4
Sick sinus syndrome 1
Cardiogenic shock 1
Myasthenia gravis 1
Multiple Sclerosis 3
Parkinson's 1
Delirium 1
Seventh Nerve Palsy 4
Join pain 47
Knee 23
Hip 4
Hand/wrist 6
Neck/Back 7
Diffuse 1
Ankle 2
Elbow 1
Shoulder 3

Table 3
Comparison of patient groups: LD vs. without LD.

	LYME DISEASE PSESENT	%	LYME DISEASE ABSENT	%	P- value
	N = 181		N = 228		
Men	169	93.4	211	92.5	0.75
Age (median)	65		66		0.236
Caucasian	173	95.6	211	92.5	0.23
Black	7	95.6	17	7.5	
Other	1	0.6	3	1.3	
History of Diabetes	34	18.8	47	20.6	0.64
Tick bite < 30 days	34	18.8	10	4.4	
Tick bite > 30 days	29	34.8	18	7.9	
Tick bite History	63	34.8	28	12.3	0.00001
EM rash	32	17.7	0		
Headache	27	14.9	19		0.036
Joint Ache/ swelling	58	32	47	20.6	0.0086
Seventh Nerve Palsy	5	2.8	4	1.8	0.49
Myalgias	34	18.8	17	7.5	0.00057
Palpitations	2	1.1	0		

Patients with early LD and EM do not need testing **to receive** treatment. C6P testing for EM was often performed in our Emergency Room. As discussed earlier, when used as a stand-alone test, the C6P is more sensitive than the current 2-tiered test for patients with early Lyme disease. Therefore, it is not surprising **that the** C6P test performed well in LD with EM, while the Western immunoblot test was negative.

Patients who were not aware of any tick bite or rash, may present several weeks or even a few months later with one of various clinical pictures **of** LD. These include aseptic meningitis, **seventh nerve** palsy, heart block, and arthritis. Also, patients unaware of any tick bite or rash can present with a combination of symptoms, but without objective signs, such as persisting vague fatigue, arthralgias or myalgias, paresthesias, and neurocognitive dysfunction that could be due to LD. Such patients frequently undergo various tests and numerous evaluations, including rheumatology, neurology, and infectious disease consultations. **Occasionally, testing (or re-testing) for LD is requested by patients who put pressure on their physicians as to "not miss" the diagnosis**. Pre-test probability dictates when a test should be ordered, and reported symptoms are thrown into the gears of clinical analysis, evaluation and deduction to produce differential diagnoses. Consequently, screening for LD is **very common** especially in our region where it can be part of the differential diagnosis in **various clinical** presentations. The positive predictive value of a test depends on prevalence of the disease. The New York State Department of Health (2013) estimated the incidence of LD per 100,000 in Suffolk county to be 41.9. Testing patients with non-specific complaints who likely don't have the disease can lead to false positive screening tests. From our review it is not surprising that C6P was falsely positive in 55 asymptomatic patients see Table 2. Nonetheless, 9 asymptomatic patients had evidence of past infection see Table 1. As the C6P test is hampered by decreased specificity, it is prone to false positive results. Molins et al. (2016) and Pegalajar-Jurado et al. (2018) showed a 4% false positive rate for the C6P in healthy blood donors from areas where LD is **nonendemic.** Therefore, the performance of C6P in our institution with a positive predictive value of 44.5% and many false positive results is not surprising.

The band at 41 kDa of the IgG immunoblot corresponds to *B. burgdorferi* **sensu stricto** flagellar protein. This band was the most observed in Lyme negative patients. This may be due to the fact this band can cross react with other bacterial flagellar proteins (Moore et al., 2016). This band was also observed in 43% of healthy controls in one study, including many persons with little or no exposure risk for LD (Moore et al., 2016).

In Johansson et al. (2017) study involving healthy blood donors sero-positivity for C6P persisted over time up to 29 months. Similarly, a few of our patients' C6P **(and persistent negative WB**) remained positive for up to five years.

Fallon et al. (2014) compared C6P assays from two commercial laboratories. The test was done on patients who were treated for LD. The C6P positivity in these laboratories was 67.6% and 62.2%. Using a 2-tiered approach combining the initial positive C6P with an IgG immunoblot, the above laboratories' positive rates returned as 40.5% and 45.9%, respectively (Fallon et al., 2014).

Kalish et al. (2001) followed the immunologic responses of the serologic tests **up to 20 years** in patients that were treated for early LD and Lyme arthritis. Antibiotic treatment for early LD appeared to dampen the antibody response and diminish the longevity of the response as only 17% of treated patients continued to have positive IgG immunoblots. In contrast, the antibody IgG levels in patients with Lyme arthritis persisted regardless of antibiotic treatment (Kalish et al., 2001).

Branda et al. (2011) **improved** the C6P specificity by using a 2-tiered enzyme immunoassay (EIA) approach consisting of whole-cell sonicate EIA followed by reflex VlsE C6P. This approach provided a higher sensitivity for early Lyme disease (61% vs. 48% for 2-tiered testing) and equivalent specificity (99.5%) to the current approach

Table 4
Antibiotic Treatment choices and outcomes.

	Lyme Disease Present (181)	%	Symptoms Resolved	Partial response	No Response	Lyme Disease Absent (228)	%
#treated	129	71.3				27	11.8
Doxycycline	110	60.8	68/81		13/81	18	7.9
Amoxicillin	8	4.4	6/7	1/7		7	3.1
Ceftriaxone	8	4.4	5/7	0/7	2/7	0	0
Augmentin	2	1.1	2/2			0	0
Dicloxacillin	1	0.6	1/1			0	0

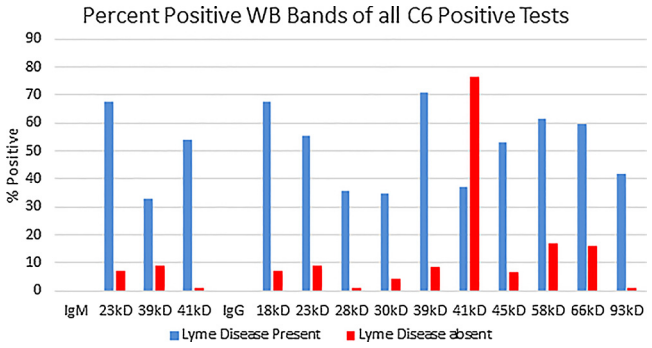

Fig. 1. Positive Western Blot Bands of all positive C6 Peptide tests.

(Branda et al., 2011).

Our study using a well-known methodology for epidemiological research is, nevertheless, subject to several potential limitations stemming from its retrospective design and experience from a single center. As our patients were not on a defined prospective clinical protocol, it is possible that not every case of LD was detected. This could be due to incomplete documentation or unrecorded information, challenge of establishing cause and effect, reviewer bias, and unknown population at risk estimate. Even though our institution has community based outpatient clinics that can be as far as 1 h from the main campus, it is likely that Veterans may have been treated for LD in different health systems. In addition, actual cases of LD with negative immunoblot may have been missed in our cohort as no follow up immunoblot testing was performed.

5. Conclusion

LD is endemic in Long Island, New York, and our Veterans were afflicted by various presentations of the disease. Current methodologies for sero-diagnosis of LD have pitfalls and can lead to misdiagnosis and inappropriate treatments. Using C6P for screening also can be hampered by false **positive** tests. When considering testing, clinicians must take into account the patient's history, timeline of symptoms, and pretest probability to accurately order and interpret the test **results**. Infectious diseases specialists can play a paramount role in education, disease prevention and provide expert advice and guidance in interpretation of laboratory results and clear indications for LD antibiotic treatment.

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Conflict of interest for all authors

None

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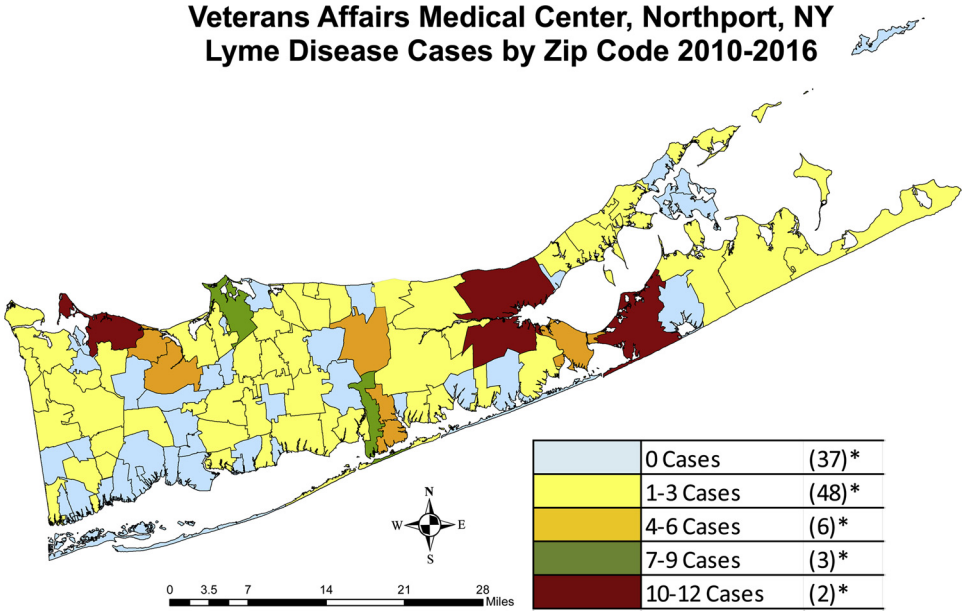

Fig. 2. Lyme disease cases by zip code among US Veterans, Suffolk County, Long Island, NY, 2010–2016.

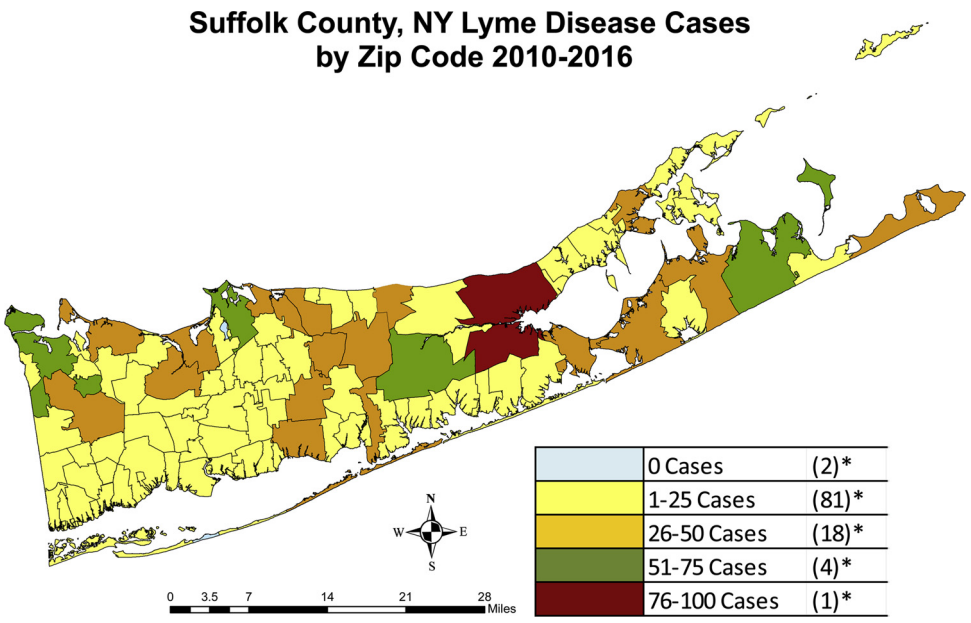

Fig. 3. Lyme disease cases by zip code, Suffolk County NY, Department of Health, 2010–2016.

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