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Divergent opinions of proper Lyme disease diagnosis and implications for children co-morbid with autism spectrum disorder

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ABSTRACT

This paper proposes that some children with an autism spectrum disorder (ASD) in the United States have undiagnosed Lyme disease and different testing criteria used by commercial laboratories may be producing false negative results. Two testing protocols will be evaluated; first, the Centers for Disease Control (CDC) and Infectious Disease Society of America (IDSA) approved two-tiered Enzyme Immunoassay (EIA) or Immunofluorescence Assay (IFA) followed by an IgM and/or IgG Western Blot test. Second, a clinical diagnosis (flu like symptoms, joint pain, fatigue, neurological symptoms, etc.) possibly followed by a Western Blot with a broader criteria for positive bands [1]. The hypothesis proposes that the former criteria may be producing false negative results for some individuals diagnosed with an ASD. Through an online survey parents of 48 children who have a diagnosis of an ASD and have been diagnosed with Lyme disease were asked to fill out the Autism Treatment Evaluation Checklist (ATEC) before they started antibiotic therapy and after treatment. Of the 48 parents surveyed 45 of them (94%) indicated their child initially tested negative using the two-tiered CDC/IDSA approved test. The parents sought a second physician who diagnosed their child with Lyme disease using the wider range of Western Blot bands. The children were treated with antibiotics and their scores on the ATEC improved. Anecdotal data indicated that some of the children achieved previously unattained developmental milestones after antibiotic therapy began. Protein bands OSP-A and/or OSP-B (Western Blot band 31) and (Western Blot band 34) were found in 44 of 48 patients. These two bands are so specific to *Borrelia burgdorferi* that they were targeted for use in vaccine trials, yet are not included in the IDSA interpretation of the Western Blot.

Autism

Autism is a developmental disorder that appears in the first 3 years of life. It is a physical impairment linked to abnormal biology and chemistry that affects typical development of social and communication skills [2]. Over the last twenty years the reported prevalence of autism has increased over 600% [3]. The increased awareness has led to a classification of Autism Spectrum Disorders [ASDs]. ASDs are a group of developmental disorders including, Autism Disorder, Asperger Syndrome, and Pervasive Developmental Disorder – Not Otherwise Specified [PDD-NOS] [4,5]. The latest data from the CDC indicates 1 in every 68 children will be diagnosed with an ASD [4].

Lyme disease

Lyme disease is a multisystemic illness caused by the spirochete bacteria *Borrelia burgdorferi* [Bb]; it is the most common vector born disease in the United States [6]. The most common mode of transmission of Lyme disease is through the bite of an infected Ixodes Scapularis tick (also known as a deer tick) [6]. Misdiagnosis of initial symptoms of Lyme disease and delayed treatment can lead to debilitating chronic illnesses with musculoskeletal, cognitive, and neuropsychiatric impairments [7]. Children who have been undiagnosed and later found to have Lyme disease have displayed decreased reading comprehension and handwriting skills, impaired speech fluency, attention deficit behavior, hyperactivity, withdrawal from activities with peers, inability to perform at grade level, obsessive compulsive behavior, anxiety, mood swings, dyslexic-like behaviors, sensitivity to light and sound, and inability to manage frustration [8]. Lyme disease has been called "The Great Imitator" because

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infected individuals often present neurological and physical symptoms that are similar to other disorders [9].

Diagnosing lyme disease

There is a division of opinions between physicians on how to properly diagnose Lyme disease. Some physicians follow guidelines published by the Infectious Disease Society of America (IDSA), while others follow guidelines published by International Lyme and Associated Disease Society (ILADS). Both guidelines are peer reviewed and evidence based [10]. The IDSA guidelines are recommended to physicians by both the CDC and the Food and Drug Administration (FDA).

According to the CDC the proper way to diagnose Lyme is:

> Signs and symptoms (flu like symptoms, joint pain, fatigue, neurological symptoms) and a history of possible exposure to infected blacklegged ticks
> Laboratory blood tests are helpful if used correctly and performed with validated methods. Laboratory tests are not recommended for patients who do not have symptoms typical of Lyme disease. Just as it is important to correctly diagnose Lyme disease when a patient has it, it is important to avoid misdiagnosis and treatment of Lyme disease when the true cause of the illness is something else [6].

The CDC recommends the following protocol for laboratory testing:

> The first required test is the Enzyme Immunoassay (EIA) or Immunofluorescence Assay (IFA). If this test yields negative results, the provider should consider an alternative diagnosis; or in cases where the patient with has had symptoms for less than or equal to 30 days, the provider may treat the patient and follow up with a convalescent serum. If the first test yields positive or equivocal results, two options are available: (1) If the patient has had symptoms for less than or equal to 30 days, an IgM Western Blot is performed; (2) if the patient has had symptoms for more than 30 days, the IgG Western Blot is performed. The IgM should not be used if the patient has been ill for more than 30 days [6].

Physicians who follow ILADS guidelines use a clinical diagnosis of neurological symptoms, exposure to an area endemic for ticks, joint pain, inflamed lymph nodes and family history of Lyme or autoimmune disorders [11]. ILADS physicians also use laboratory testing, but do not always determine a patient negative for Lyme if the first-tier test comes back negative (EIA or IFA) [11]. Many times a Western Blot is performed and a broader view of the results are evaluated. There are nine known Bb species specific Western Blot antibodies (bands): 18, 23, 31, 34, 37, 39, 83 and 93 [12]. Only one of these Bb genus specific bands is needed to confirm that there is lab evidence of exposure to the Bb spirochete and can confirm a clinical diagnosis of Lyme disease [11]. CDC Western Blot IgG surveillance criteria include 18, 23, 30, 37, 39 and 93 and exclude bands 31, 34 and 83, even though bands 31 and 34 are so specific to Bb they were targeted for use in Lyme vaccine trials [13].

Controversy with laboratory testing

The CDC cautions against using unapproved testing:

> CDC and the Food and Drug Administration (FDA) have become aware of commercial laboratories that conduct testing for Lyme disease by using assays whose accuracy and clinical usefulness have not been adequately established. Health-care providers are reminded that a diagnosis of Lyme disease should be made after evaluation of a patient's clinical presentation and risk for exposure to infected ticks, and, if indicated, after the use of validated laboratory tests [14].

The CDC also states

> Surveillance case definitions establish uniform criteria for disease reporting and should not be used as the sole criteria for establishing clinical diagnoses, determining the standard of care necessary for a particular patient, setting guidelines for quality assurance, or providing standards for reimbursement [15].

However many use the CDC surveillance criteria as a diagnostic criteria.

The two-tiered testing model is the only laboratory testing approved by the CDC and FDA, but the sensitivity of the test has been scrutinized. The sensitivity of the two-tier test system has been estimated to be 44%–56% when standard commercial Lyme testing was evaluated in clinical practice [1,10,16–22]. In a molecular diagnostic study, the sensitivity of this testing approach may be as low as 7.5% [23]. Some reasons for the false negative test result include: specimen improperly handled; test run too soon (antibodies to Bb have not formed); Western Blot does not test for all antigens; immune suppression by recent antibiotics; lab tests are standardized for early, not late stage Lyme disease; antibodies may decline over time; antibodies are bound up in immune complexes (tests detect only free antibodies); spirochetes may be hidden or dormant in a cell deficient wall [13].

The outcomes of the two-tiered testing has raised concerns with physicians about its validity. Recently Virginia and Maine (United States) passed bills which require all doctors in their state to inform their patients that a negative outcome on the two-tiered test does not conclusively rule out infection of Bb [24,25].

Lyme/Autism connection

A study of was conducted by Dr. Garth Nicolson in 2003 where 20% of the children ($n = 48$) diagnosed with an ASD came back positive for Lyme disease [26]. A similar study was conducted by Dr. Aristo Vojdani [27] and 22% of the ASD patients ($n = 54$) he tested came back positive. Kuhn et al. [19] evaluated five children diagnosed with an ASD and Lyme disease before and after they were treated with antibiotic therapy. All five children in the study showed improvement on the ASD evaluation tool used to assess them after they completed treatment [19]. In all of the published studies the children were considered positive using a clinical diagnosis and a broader interpretation of the Western Blot [26,19,27]. In a personal interview with an assistant of Dr. Charles Ray Jones, a pediatric physician who exclusively treats Lyme disease, it was claimed that 50% of his patients who have been diagnosed with an ASD have come back positive for Lyme disease. He also claimed that all of his patients' symptoms improve with antibiotic therapy. In a recent study Ajamian et al. [28] evaluated 120 children, 70 with ASD and 50 healthy controls for Lyme using the two-tiered Lyme test. Using the CDC approved two-tiered testing protocol 0/120 children tested positive.

Hypothesis

Shortly after the Ajamian et al. study was published numerous medical media websites and publications reported headlines akin to: "Lyme/Autism theory debunked." The mentioned study followed CDC and FDA approved laboratory guidelines for Lyme

disease testing and it was conducted with an appropriate control sample [28]. We believe it is inappropriate to make a definitive statement about the connection between Lyme disease and Autism without using clinical criteria and other diagnostic tests. Our hypothesis is that some patients with an ASD who test negative using the two-tiered laboratory Lyme test are actually receiving a false-negative and their autistic symptoms will improve with proper antibiotic treatment. To test this we sent an online survey to parents of 48 children who claimed to have a child with a diagnosis of Lyme disease and an ASD. To be considered for the survey the parents had to submit the child's official diagnosis of an ASD from a licensed psychologist and a laboratory test showing some sign of Bb infection. In the initial questionnaire we asked if their child met the CDC two-tiered guidelines (6% [3/48] responses reported that they met the criteria). The parents of the other 45 children took their child to another physician who diagnosed them with Lyme disease using the broader interpretation of the Western Blot and clinical criteria like neurological symptoms, fatigue, relapse of flu like symptoms, and sensitivity to light and sound.

To determine if the treatment did alleviate any autistic symptoms the parents filled out the Autism Treatment Evaluation Checklist (ATEC) twice. First, they completed the ATEC describing their child's symptoms before they started antibiotic therapy and second after treatment finished.

The ATEC was developed in 1999 to help researchers evaluate the effectiveness of various treatments for children with autism and adults and to help parents determine if their children benefit from a specific treatment. It is a one-page form designed to be completed by parents, teachers, or caretakers. It consists of 4 subtests: I. Speech/Language Communication (14 items); II. Sociability

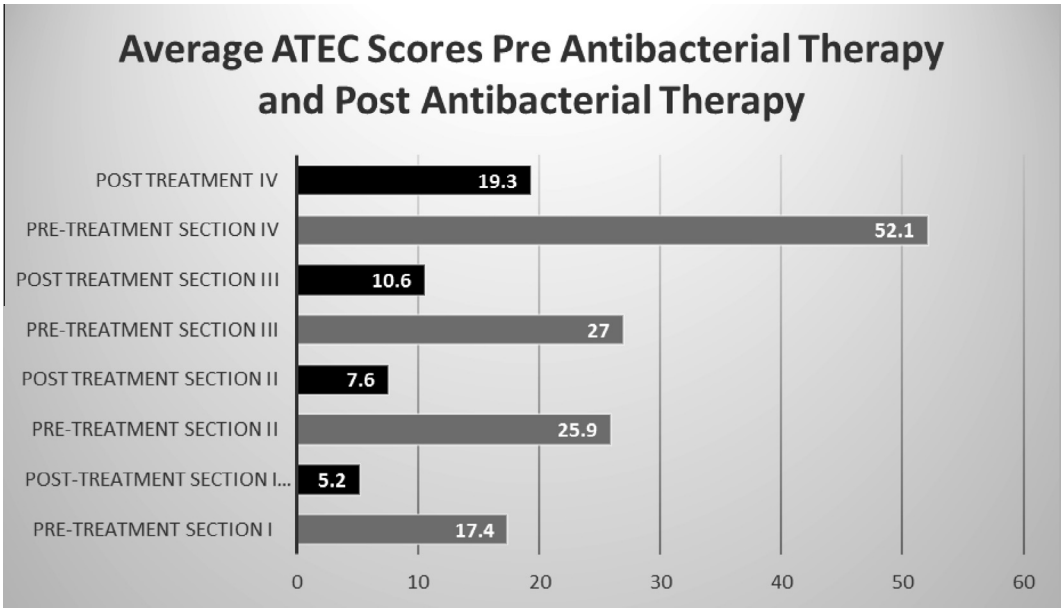

Fig. 1. Pre and post ATEC scores reported by the parents in the study. The subsets of the ATEC are: Section I – Speech/Language/communication, Section II – Sociability, Section III – Sensory/Cognitive Awareness, Section IV –Health/Physical/Behavior.

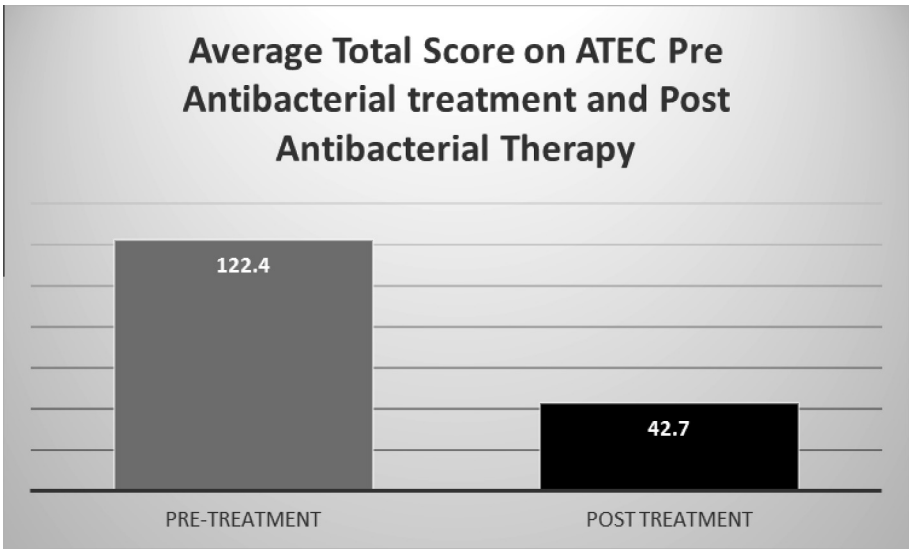

Fig. 2. Total pre and post ATEC scores reported by the parents in the study. A score of 104–180 is considered in the severe range. A score of 30 or below is considered mild.

(20 items); III. Sensory/Cognitive Awareness (18 items); and IV. Health/Physical/Behavior (25 items).

When the parents complete the ATEC they select a zero if the phrase is not true, a one if it is sometimes true, and a two if it is very true; the lower the score the less severe the autistic symptoms. When the subset ratings are added a total score of 180 is possible. Scores closer to 180 would indicate a severe case of autism symptoms and a score less than 30 indicates a mild case of autistic symptoms. The ATEC is not copyrighted and may be used free of charge by any researcher [29]. The Autism Research Institute examined the reliability of the ATEC by conducting a split-half reliability test on over 1300 completed ATECs. The internal consistency reliability was high (.94 for the Total score) [29].

Empirical data

All 48 of the children's scores on the ATEC survey improved with antibiotic therapy (see Fig. 1). The overall mean score of the children moved from a classification of severe-autism to close to mild-autism (Fig. 2). Parents were also given the opportunity to explain in greater detail any changes they observed during treatment, some responded with anecdotal data (Fig. 3). The parents of the children in the study indicated that no other educational or biomedical therapies were changed during the period they treated their child's Lyme disease infection.

Consequence of the hypothesis and discussion

A large majority of the children in the survey would not have received antibiotic therapy if their physician followed the two-tiered IDSA/CDC recommended testing [93.8% (45/48)]. The CDC's Morbidity and Mortality Weekly Report (MMWR) claimed: "We do not recommend skipping the first test and just doing the Western Blot. Doing so will increase the frequency of false-positive results and may lead to misdiagnosis and improper treatment [14]." Yet, the physicians who treated the children in the survey

"Regained the ability to speak"
"Spoke first word three weeks after we started antibiotics."
"Reduced anxiety in public. Better sleep. Increased playtime with other kids. Social gains. Increased language."
Was completely non-verbal, now can speak in full sentences
"Before we started treatment my child needed round the clock supervision at school with a full time aid. Now he is in a regular education classroom with his peers almost the entire day with no assistance."
"My child use to self-injure himself (slap himself in the face, hit his head against the wall). All of those behaviors stopped once we treated his Lyme."

Fig. 3. A few examples of anecdotal data collected from the survey. Above are responses to the question: Did your child achieve any significant developmental milestones during treatment? Six out of forty eight participants (12.5%) responded "No.".

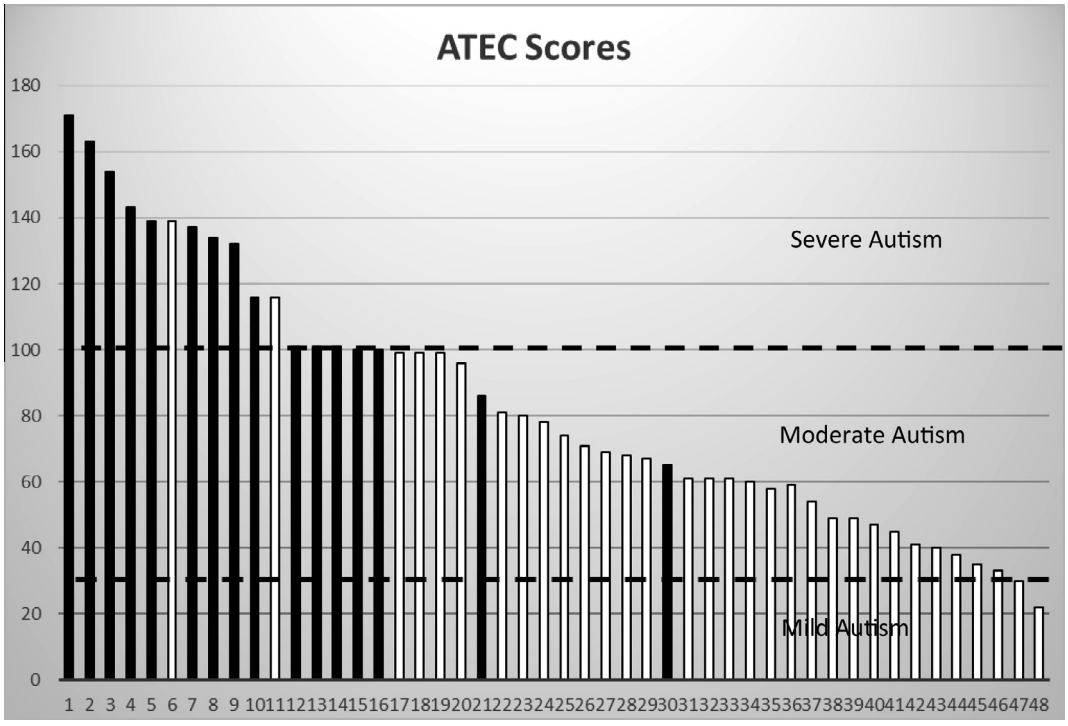

Fig. 4. The pre-treatment ATEC scores of all 48 children in the study. The scores in black indicated a positive result for OSP B. The scores in white had other positive bands on their Western Blot but not OSP B.

did exactly what the MMWR warned against and the benefit to their patients was evident in the ATEC scores and the anecdotal data. The results of the study raise questions about the validity of the commercially approved IDSA/CDC Lyme disease test. If the parents of the children in the study had not pursued a second opinion from a physician who followed ILADS guidelines their child would have not received a Lyme disease diagnosis and they would have not received antibiotic therapy. The improved ATEC scores clearly indicate that the antibiotics had a positive impact on the welfare of the patients. These antibiotics would not have been prescribed if the treating physician followed CDC/IDSA guidelines. It is fair to questing if the prescribed antibiotics provided an assuaging effect on the patient's autistic symptoms due to an unidentified pathogen. But, the presence of multiple Bb specific protein bands in the patient's Western Blot suggest the patients improved health was due to the treatment of their Lyme disease. In the Ajamian et al. study none of the 70 children with autism in the study were treated with antibiotics, so it is not possible to determine if antibiotics would have been beneficial to them. This is not to suggest that antibiotics should be given to children with Autism who show no signs of Lyme disease, instead a deeper look at the diagnostic criteria should be considered to rule out infection.

Further evaluation of the data acquired from the survey indicated the presence of OSP-A and OSP-B in the children's Western Blot was prevalent. Of the 48 surveys collected 44 (91%) received a positive indication of said bands. These two bands are so specific to Bb that they were targeted for use in vaccine trials, yet are not included in the CDC interpretation of the Western Blot [29,13]. None of the parents in the survey indicated that anyone in their family had received a Lyme vaccine. In the Ajamian et al. [28] study only 5 of the 70 children were tested using the Western Blot and OSP-A and OSP-B were not included in the evaluation.

Further dissemination of the data revealed that nearly all of the children who received an ATEC score of severe-autism had band OSP-B present on their Western Blot (Fig. 4). According to the results of the survey, 16 of the 48 children had a score of severe-autism. Of those 16 children 14 (88%) tested positive for OSP-B. Of the remaining 33 children who's scores were in the moderate to mild range only 2 children (6%) tested positive for OSP-B. This data could be significant because, as indicated earlier, OSP-B is not typically tested for in commercially available Lyme disease tests. The OSP-B band could be a vital point of interest for future researchers investigating the Lyme/Autism connection. Physicians who have a patient with an ASD that who are considering a Lyme disease diagnosis should find a laboratory that tests for OSP-B before ruling out the infection.

If this small scale study is any indication of what may be causing or exacerbating the children's autistic symptoms it could mean a percentage of the autism community is actually suffering from Lyme disease and some of those symptoms could be alleviated with proper testing and treatment. We believe it is advantageous for parents who have a child diagnosed with an ASD to seek out a physician who does not strictly follow the two-tiered laboratory criteria to diagnose Lyme disease.

Dr. Bransfield has testified as an expert in cases involving Lyme disease.

Limitations of this study include no physical interaction or observation of the children and only using the parents survey results in the study. The parents did not indicate what type of antibiotics or antibacterial therapy was used to treat their child was used or the duration of its use.

Conflict of interest statement

None.

References

[1] Steere AC, McHugh G, Damle N, Sikand VK. Prospective study of serologic tests for Lyme disease. Clin Infect Dis 2008;47:188–95.
[2] Ma B, Christen B, Leung D, Vigo-Pelfrey C. Serodiagnosis of Lyme borreliosis by Western immunoblot: reactivity of various significant antibodies against *Borrelia burgdorferi*. J Clin Microbiol 1992;30:370–6.
[3] Autism Speaks. Autism Speaks: Science Answers. <http://blog.autismspeaks.org/2010/10/22/got-questions-answers-to-your-questions-from-the-autism-speaks%E2%80%99-science-staff-2/>. [Retrieved 6/30/2013].
[4] Centers for disease Control. Autism. <http://www.cdc.gov/ncbddd/autism/index.html>. [Retrieved 6/30/2013].
[5] National Institute of Mental Health. Autism Spectrum Disorders: Persuasive Developmental Disorders. 2008. (NIH Publication No. 08-5511). Washington, DC: U.S.
[6] Centers for Disease Control and Prevention. Lyme Disease. <http://www.cdc.gov/ncidod/dvbid/lyme/index.htm>. [Retrieved 6/10/2013].
[7] Cameron, D. Treatment delay as a risk factor for treatment failure in Lyme disease. In: 16th International Scientific Conference on Lyme Disease and Other Tick-borne Disorders, 2003, Hartford, CT, June 7–8.
[8] Hamlen RA, Kilman DS. Lyme disease: etilogy, neuropsychological sequelae, and educational impact. Nat Assoc School Psychol 2007;35(5):34–8.
[9] Bransfeld RC. Lyme disease, comorbid tick-borne diseases, and neuropsychiatric disorders. Psychiatric Times 2007;24:29–32.
[10] Stricker RB, Johnson L. Lyme wars: let's tackle the testing. Br Med J 2007;335:1008.
[11] Cameron DJ, Gaito A, Harris N, Bach G, Bellovin S, Bock K, et al. Evidence-based guidelines for the management of Lyme disease. Expert Rev Anti-Infect Ther 2004;2(1).
[12] Tylewska-Wierzbanowska S, Chmielewski T. Limitation of serological testing for Lyme borreliosis: evaluation of ELISA and Western blot in comparison with PCR and culture methods. Wien Klin Wochenschr 2002;114(13–14):601–5.
[13] Hilton E, Devoti J, Sood S. Recommendation to include OspA and OspB in the new immunoblotting criteria for serodiagnosis of Lyme disease. J Clin Microbiol 1996;34(6):1353–4.
[14] Centers for Disease Control. Notice to readers: caution regarding testing for Lyme disease. Morbidity and Mortality Weekly Report. CDC Surveillance Summary. 2005. 54:125.
[15] Centers for disease Control. Resource for Clinicians. <http://www.cdc.gov/lyme/healthcare/clinicians.html> [Retrived 7/22/2013].
[16] Bacon RM, Biggerstaff BJ, Schriefer ME, Gilmore Jr RD, Philipp MT, Steere AC. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with 2-tiered testing using whole-cell lysates. J Infect Dis 2003;187:1187–99.
[17] Engstrom SM, Shoop E, Johnson RC. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J Clin Microbiol 1995;33(2):419–27.
[18] Johnson L, Stricker RB. Treatment of Lyme disease: a medicolegal assessment. Expert Rev Anti-Infect Ther 2004;2(4):533–57 [PMID: 15482219].
[19] Kuhn M, Branseld R, Graves S, Harris S. Long term antibiotic therapy may be an effective treatment for children co-morbid with Lyme disease and autism spectrum disorder. Med Hypothesis 2012;78(5):606–15. http://dx.doi.org/10.1016/j.mehy.2012.01.037.
[20] Ledue TB, Collins MF, Craig WY. New laboratory guidelines for serologic diagnosis of Lyme disease: evaluation of the two-test protocol. J Clin Microbiol 1996;34(10):2343–50.
[21] Schmitz JL, Powell CS, Folds JD. Comparison of seven commercial kits for detection of antibodies to *Borrelia burgdorferi*. Eur J Clin Microbiol Infect Dis 1993;12:419–24.
[22] Trevejo RT, Krause PJ, Sikand VK, Schriefer ME, Ryan R, Lepore T, et al. Evaluation of two-test serodiagnostic method for early lyme disease in clinical practice. J Infect Dis 1999;179:931–8.
[23] Santino I, Berlutti F, Pantanella F, Sessa R, del Piano M. Detection of *Borrelia burgdorferi* sensu lato DNA by PCR in serum of patients with clinical symptoms of Lyme borreliosis. FEMS Microbiol Lett 2008;283:30.
[24] An Act To Inform Persons of the Options for the Treatment of Lyme Disease. Maine Code Ann. MRSA §1645, sub-§3. (2013).
[25] Lyme Disease Testing Disclosure Act. Virginia Code Ann.§ 54.1-2963.2. (2013).
[26] Bransfield RC, Wulfman JS, Harvey WT, Usman AI. The association between tick-borne infections, Lyme borreliosis and autism spectrum disorders. Med Hypotheses 2008;70(5):967–74.
[27] Vojdani A. Immunology of Lyme disease and associated disorders. In: LIA Conference, 2007.
[28] Ajamian M, Kosofsky BE, Wormser GP, Rajadhyaksha AM, Alaedini A. Serologic markers of Lyme disease in children with autism. J Am Med Assoc 2013;2013(309):1771–2.
[29] Autism Research Institute. Evaluation of the Autism Evaluation Treatment Checklist. <http://www.autism.com/index.php/ind_atec_report>. [Retrieved 6/18/2013].