14th International Scientific Conference on Lyme Disease & Other tick-Borne Disorders:

Diagnosis, Treatment, & Research Update

April 21 - 23, 2001 Farmington, CT, USA

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Disease _____

Prevention & Basic Science
- __ Ecology, Entomology
- __ Vaccine
- __ Animal models
- __ Microbiology
- __ Pathogenesis
- __ Pathology
- __ Other:

Patient Management
- __ Clinical manifestations
- __ Laboratory diagnosis
- __ Early disease management
- __ Late disease management
- __ Chronic disease management
- __ Other:

AGGRESSIVENESS & TICK-BORNE DISEASES

The patient is a 14 year old White male with a history of Lyme and other infectious diseases, and a tendency towards aggressive behavior. The patient may have had congenital exposure to Lyme disease, there is a history of multiple tick bites and there were bull's eye rashes on two separate occasions – age 6 and 8. His symptoms include difficulty sustaining attention, being easily distracted by frustration, olfactory hyperacusis, stuttering, intrusive images, hypnagogic hallucinations, apathy, decreased frustration tolerance, sudden abrupt mood swings, disinhibition, exaggerated startled reflex, explosive anger, violent outbursts, being accident prone, decline of school performance, disruptive behavior, depression, not being well rested in the morning, insomnia, hypersomnia during the day, intolerance to heat, conjunctivitis, dizziness, motion sickness, joint pain crepitations, sore throats, and irritable bowel. These symptoms progressively increased over time. The aggressiveness tendencies were particularly troublesome as the patient would attempt to choke his mother to death, throw things around the house, knock over furniture, and kick and punch holes throw walls and doors. Frequent police intervention was needed. There is a lack of empathy and a troubling lack of remorse for his behavior.

Positive findings on laboratory reports include: Lyme IgM, Lyme IgG, Lyme PCR urine, HHV-6, Bartonella, stealth virus culture, and Bartonella in spinal fluid. PET Scan demonstrated a slightly heterogeneous uptake of radeotracer with foci of mild hypermetabolism in the left parietal & bilateral medial temporal lobes, an MRI was normal. The patient was treated with a number of different psychotropic and antimycrobial strategies. He is currently showing a partial response to a combination of Famvir 500 mgs. 3 times a day, Claforan 3 grams twice a day, Zithromax 500 mgs. twice a day, Risperdal 5 mgs. twice a day, and Concerta 18 mgs. in the morning with 5 mgs. of methyphynlidate in the late afternoon. Whenever the antibiotics are stopped, the patient becomes quite aggressive. This is especially true with Famvir which results in increasing irritability and aggressiveness within 24 hours whenever the medication is discontinued.

DISCUSSION:

This case demonstrates the association that is sometimes seen with tick-borne diseases and aggressive behavior. Further research is needed in this area.

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DISEASE Chronic Lyme Disease

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- __ Ecology, Entomology
- __ Vaccine
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- ✓ Pathogenesis
- __ Pathology
- __ Other:

PATIENT MANAGEMENT
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- __ Late disease management
- __ Chronic disease management
- __ Other:

RECOVERY OF LYME SPIROCHETES BY PCR IN SEMEN SAMPLES OF PREVIOUSLY DIAGNOSED LYME DISEASE PATIENTS

Dr. Gregory Bach, D.O., P.C.

OBJECTIVE:

Lyme disease, being a spirochete with pathology similar to syphilis, is often found difficult to treat due to the spirochete invading sanctuary sites and displaying pleomorphic characteristics such as a cyst (L-form). Because a significant portion of sexually active couples present to my office with Lyme disease, with only one partner having a history of tick exposure, the question of possible secondary (sexual) vector of transmission for the spirochete warrants inquiry. Additionally, sexually active couples seem to have a marked propensity for antibiotic failure raising the question of sexually active couples re-infecting themselves through intimate contact.

METHODS:

Lyme spirochetes/DNA have been recovered from stored animal semen (Br Vet J. 1995 Mar-Apr,151(2):221-4). Recovery of spirochete DNA from nursing mother's breast milk and umbilical cord blood by PCR (confirmed by culture/microscopy), have been found in samples provided to my office.

RESULTS:

Surprisingly, initial laboratory testing of semen samples provided by male Lyme patients (positive by western blot/PCR in blood) and the male sexual partner of a Lyme infected female patient were positive approximately 40% of the time. PCR recovery of Lyme DNA nucleotide sequences with microscopic confirmation (Infection. 1998 Nov-Dec;2626(6):364-7, PMID: 9861561; UI: 99078554) of semen samples yielded positive results in 14/32 Lyme patients (13 male semen samples and 1 vaginal pap). All positive semen/vaginal samples in patients with known sexual partners resulted in positive Lyme titers/PCR in their sexual partners. 3/14 positive semen patients had no or unknown sexual partners to be tested.

These preliminary findings warrant further study. Current a statistical design study to evaluate the possibility of sexual transition of the spirochete is being undertaken. Our laboratory studies confirm the existence of Lyme spirochetes in semen/vaginal secretions. Whether or not further clinical studies with a larger statistical group will support the hypothesis of sexual transmission remains to be seen. A retrospective clinical study is also underway. We are reviewing the medical records, collecting semen samples of previously treated Lyme positive patients as well as collecting vaginal and semen samples of Lyme positive patient's blood. Male/female patients who were previously diagnosed with current and previously treated Lyme disease are being asked to provide semen, pap and blood samples for extensive laboratory testing

CONCLUSION:

With the initially impressive data, we feel the subsequent statistical study on the sexual transmission of the Lyme spirochete will illuminate a much broader spectrum of public health concerns associated with the disease than the originally accepted tick borne vector.

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CORRECTION OF PERSISTENT CARDIAC ABNORMALITIES WITH PROLONGED ANTIMICROBIAL TREATMENT IN A TEENAGER WITH CHRONIC TICK BORNE DISEASES.

Authors: Gregory Bach, D.O., Danzel Pollack, M.D., F.A.C.C., Harold Smith, M.D.,F.A.C.E.P., Private and research practice – Lansdale, PA.

OBJECTIVES:

The authors report the favorable clinical progress of an adolescent with CDC confirmed tick borne diseases with multiple cardiac abnormalities through diligent antimicrobial treatment and with literature review drawn attention to, not only the benefit, but the need for more cardiac clinical pursuit in other patients with chronic tick borne diseases.

METHODOLOGY:

Review of the medical literature pertaining to Borrelia Burgdorferi infection and cardiac manifestations and case report of a patient investigated by standard serologies, PCR, DNA testing, EKG, and stress EKG studies, repeat holter monitoring, and echocardiography. Sample studies on poster presentations.

RESULTS:

Pathologist Paul Duray reported the involvement of all layers of the heart with chronic Bb inflammatory injuries. (1) Clinically, studies have minimized concern about long term deleterious cardiac effects, even questioning antibiotic need. (2,3,4,5,6,7), raised concern (8,9,10,11), or claimed serious consequences (12,-,21). A 13 year old male presented with headache, chest pain, palpitations, fatigue and cognitive deficits which had accelerated over three weeks. Remote history positive for biopsy confirmed tick bite exposure and 10 day amoxacillin rx. Now ELISA and Western Blot positive (31,34) IgM for Bb infection and tier positive for Ehrlichia and Babesia. PCR, DNA and Bb at MDL. EKG sinus bradycardia. Holter monitor sinus node dysfunction with sleep rates alternating to 150 or 40 and T wave inversion at rapid rates. Echo normal. Stress EKG, exercise induced rapid rate compared to sleep associated with less T wave change. Treated with long term oral tetracyclines, Mepron/artomesia, and macrolides. Nardir three months lateral with new mitral valve regurgitation and diffuse T wave inversion in resting EKG associated with profound fatigue. Negative CPK and troponin. Routine blood cultures negative. Continued antimicrobial protocols. Improved over ensuing 6 mos with normal mitral valve but mild pulmonary regurgitation, near normal holter histogram, and minor T wave changes at faster rates. Athletic and academic performance excellent. PCR, DNA still positive.

CONCLUSION:

Sinus node dysfunction with bradyarrhythmias are commonly described during Bb infection. Holter monitoring can display evening autonomic dysregulation and inability for cardiac recovery during sleep in tick borne infections. Both occurred in this patient. Vagal and postural maneuvers can demonstrate peripheral autonomic nerve dysfunction as an extrinsic etiology and be followed. Normal wall motion, cardiac enzymes, and stress EKG performance indicate lack of serious contractile dysfunction or need to biopsy. Mitral valve dysfunction may be expected to occur more commonly in Borreliosis than recognized and may set the stage for secondary later bacterial co-colonization. Pulmonary regurgitation may be a harbinger of future pulmonary hypertension if not monitored and be an explanation for the dyspnea reported in LD. The overall steady improvement of the case report indicates that prolonged oral therapy can be effective and that IgM titers correlate with new modulation of antigen presentation in a chronic infection. The authors in recognizing prolonged cardiac manifestations of tick borne diseases and end points to effective therapy. Although long term cardiomyopathies and valvular injury are not yet adequately studied, the risk and damage to the patient are significant enough to begin following patients such as the above report thoroughly with a cardiologist well read in tick borne diseases.

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REVERSAL OF SEVERE CONGENITAL MENTAL RETARDATION SECONDARY TO LYME DISEASE AND BABESIOSIS WITH I.Q. IMPROVING FROM 63 TO 107

By Gregory P. Bach, D.O.
(Edited by Virginia Shear, M.D.)

OBJECTIVE:

To present a tape study (with video documentation) to support the effective reversal of severe congenital mental retardation with treatment protocols for Lyme disease and Babesiosis.

METHODS:

The patient was diagnosed and treated in a Lyme specialized practice. A 6.5-year-old boy first presented to this Southeastern Pennsylvania practice office on 8/6/99. The patient was classified as "severely retarded" with a verbal I.Q. of 51 and a non-verbal I.Q. score of 80 (below average). The patient's overall I.Q. score was 63. At the time his mother told us that the child was in a special needs program in which he was going to be trained only for the ability to clothe himself and tie his shoes. The patient presenting symptoms were cyclic fever, fatigue in the afternoon, marked irritability, bladder dysfunction, inability to achieve potty training until the age of four and a half years, and cognitive abnormalities of confusion, difficulty thinking and decreased concentration. During the patient's birthing he experienced significant bradycardia. At the time of examination he was only able to verbalize by "parroting". For example if he was asked "What is your name?" he would repeat back "What is your name?" He also suffered from severe mood swings. There was no history of operations or allergies. He was not receiving any medications. On family history it was noted that his mother, age 40, and his father, age 42, both being treated for concurrent Lyme disease and Babesiosis. They resided in a Huntington County, New Jersey, an area very endemic for Lyme disease and its co-infections. Mother's profession involved working with animals. Both parents are of high intelligence. The patient had a sister age 7, who suffered similar problems and who had been classified as mildly retarded with and I.Q. score of 90. On physical exam the patient had a sixth cranial nerve palsy. An EM rash, greater than 5 cm. was noted at the base of his occiput region. The patient's vital signs were 94/60; temperature 96; pulse 80. Ear, nose and throat exam was within normal limits. His heart showed a regular rate and rhythm. No abdominal masses were noted; there was no abnormal signs in evaluation to his extremities nor were there any focal deficits except for the sixth cranial nerve palsy. The patient's CBC and SMAC were normal. The patient was negative for thyroid disease, negative for arthritis, negative for lupus, gout, syphilis, hepatitis A, B and C. The showed a vaccination level for hepatitis B at 19%. The patient first tested positive by PCR for Babesiosis. The patient was negative for rickettesial diseases, negative for human granulytic Ehrlichiosis and negative for human monocytic Ehrlichiosis. The patient's ELISA was read negative and 0.36. His Western Blot showed double Kd. Band #41 and IgM Western Blot showed an insignificant band at 37 Kd. The patient's blood did show a second positive test for Babesiosis, IgM, IFA of 1:40. The patient was placed on Ceftin 250 mg. per 5 cc, 3 tsp. PO daily and his test was repeated in three weeks time. At this point his Western Blot was rechecked and he came back equivocal with a P#39 Kd. IgG Western Blot changed with a positive band at Kd #41 an' now developing a Kd #31 band. The patient's chemistries remained within normal limits. The patient's mother reported he had been making tremendous strides, increased vocalization, increased eye contact. He also increased his height by one inch and had gained four and a half pounds within 21 days. In his follow up a month later, the patient had grown another inch and his mother reported that the patient had said to her "I'm happy". Mother was getting reports from school that his work performance was much improved. The patient was still on Ceftin 250 mg. per 5 cc. 3 tsp. PO daily. His repeat IgM Western Blot

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pattern was still equivocal but becoming stronger with double bands at #31 Kd and now positive at band #39 Kd. It was startling and very satisfying to the patient and doctor alike when the patient started to write for the first time in his life. His blood was cultured and it showed appearance of a ring-like growth in many erythrocytes which were consistent with Lyme and Babesiosis. The patient's blood was sent out to a third lab on 10/26/99 and it came back positive for Lyme disease, (Borrelia Burgdorferi) by PCR showing the exact DNA of the organism. It was noted at this time that the patient had grown two and a quarter inches higher in a period of nine weeks. He was started on a second antibiotic Suprax 100 mg. per 5 cc. two tsp. PO q d. The patient was seen on the next office visit on 11/19/99 as he was experiencing a major Herxheimer reaction. His mother stated that he had severe fatigue, he felt that he was washed out. At this time the patient was started on Mepron 750 mg. per 5 cc. ¾ tsp. PO daily for Babesiosis. The patient's CBC and SMAC remained within normal limits. The patient was then tested for Lyme urine antigen on 11/24/99 with a resultant score of 49 and 55, anything above 45 indicating a high level of infection as indicated by antigen specific for Borrelia Burgdorferi infection. The patient was followed up on 1/21/00. His mother noted that the patient was very clear cognitively and functioning at a higher level after the treatment with the Mepron had begun. The Mepron regimen lasted for three weeks one, two weeks off and three weeks on. Liver enzymes remained within normal limits. The parents started working with CJ in the areas of reading, mathematics and they increased his exercise time. By April his mother reported the patient was getting A's in spelling, whereas one year ago he could not spell at all. In June, 2000, his school mainstreamed the child for homeroom and music at normal level. He had a marked increased performance in math and spelling. On 6/23/00 the mother reported the patient was able to pronouce phonetically any written word. She stated that he was able to read billboards while riding down the highway. In the doctor's office there was a proclamation involving a congressional summary and the patient was able to read out loud verbally every word, although he did not understand everything he was reading. The patient's blood was recultured on 7/5/00 and it showed that he was still positive for Lyme spirochetes.

RESULTS:

On 10/4/00 the same child study team that obtained the original score on 3/5/99 performance scores, retested the patient. Much to everyone's shock, this patient's verbal score increased from 51 to 85. His performance I.Q. score changed from 80 to 131. The overall I.Q. change from 63 to an overall score of 107.

CONCLUSION:

Co-infections, especially Babesiosis, probably act as amplifiers for signs of Lyme disease, particular neurological ones (thereby confounding both its diagnosis and treatment). In this case, what appeared to be severe congenital mental retardation was actually able to be reversed by treating what appears to be congenital Lyme and Babesiosis. With an overall I.Q. improvement of 44 points. This by itself seems quite amazing and may be considered by some an isolated incident, however, the proof would really be to see if it could be repeated. While the truth of the matter is that the patient's sister who is also being treated for Lyme and Babesiosis now has an improved I.Q. as well. Her I.Q. went from 90 to 114 following treatment with antimicrobial therapy thus ruling a chance event. It is this authors conclusion that when patient's live in a highly endemic area for both Lyme and Babesiosis and displaying severe neurolo-ical signs including severe to mild retardation a thorough Lyme and its co-infection work up should be performed.

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THE LYME BORRELIOSIS ANTIBODY ELISA TEST – TIME TO DISCARD AN OUTDATED AND POTENTIALLY HAZERDOUS TEST.

INTRODUCTION:

The Lyme ELISA is often proposed as the screening test for confirm Lyme disease (1,2). However, a review of the scientific basis, literature, and the authors' experience regarding the Lyme ELISA reveals what other physicians have expressed – the ELISA test is not only unreliable, but also dangerous since it often fails to detect victims of Lyme Borreliosis (3).

METHODS AND MATERIALS:

1. The scientific basis for the Lyme Disease ELISA test is reviewed and reasons for the nonvalue of the ELISA test was summarized. (4)(5)(6).
2. The literature is searched for studies emblematic of the problem of seronegative Lyme disease and the ELISA test. (7,8,9,10,11,12,13,14,15,16,17,18,19,20,21)
3. The authors report the comparison of Lyme ELISA results in a population of 387 patients diagnosed as Lyme disease with additional information from Western Blot and direct test results for urine antigen, PCR, and culture and response to long term treatment.

RESULTS:

1. The ELISA test measures "free" antibody not attached to the borrelia antigens present. As a result the test misses patients who have active disease with antibody formed and correctly binding to borrelia, but who don't have enough uncomplexed antibody left over to exceed the quantitative cut off level. (4,5). Secondly, borrelia expresses different antigens at different temperatures and in different tissues such as central nervous system which do not match the serological strain tested. Third, the ELISA does not detect the wall less or cyst forms of borrelia. The ELISA cannot detect intracellular forms, suppression by antibiotic and steroid use, and measure the trophic nature of borrelia to invade immune privileged sites such as connective tissue, eye and brain. A patient's strain can differ markedly from the one lab strain used and the ELISA fails to recognize that the specificity of certain antibodies is much more important than a positive victims are excluded.
2. The authors reviewed the results of 387 patients referred to a Southeastern Pennsylvania Congressional Medical Advisor on Lyme Disease. All patients were clinically positive for Lyme disease and with additional supportive laboratory testing by Western Blot, direct urine antigen, PCR and or advanced culturing. The ELISA test for free antibodies was positive in only 33 (8.7%) of the 387 patients. These findings parallel many anecdotal case studies of missed Lyme Borreliosis by Lawrence Petrovic and Hudson (16,20,21)

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CONCLUSION:

As presently utilized, when positive, the ELISA test as part of a two tiered system is discounted and a second test, Western Blot, is advised. When negative, the test misses anywhere from 20% to 50% of the patients. In either case it is out of date with present scientific technology. But when it is negative, it is potentially dangerous (In the author's practice, the patients who suffer from Lyme disease and have "positive" ELISA tests are often screened out and treated elsewhere.) Those who have been misdiagnosed then as seronegative who persist in trying to get help are correctly diagnosed with diligent testing, and proper clinical diagnosis. The ELISA test may lead physicians to falsely conclude that the patient does not suffer from Lyme disease. All stages of Lyme disease respond to correct antibiotic treatment, and the failure to diagnose means extended suffering and a more difficult improvement for the patient. A thorough review of the scientific basis for the ELISA test, literature reporting seronegative Lyme Borreliosis and the author's extensive patient series leads to the following conclusion:

The test should be discarded and not ordered as presently utilized. Emphasis should be placed on diligent additional laboratory testing such as DNA-PCR, advanced cultures, along with Western Blot testing with updated criteria recognizing the importance of specific bands (eg. 23,31,34,39,88,93) plus the 41 flagellar marker. It is time to bring our testing into the 21st Century.

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THE REVERSAL OF SEVERE PSYCHOTIC BEHAVIOR SECONDARY TO LYME DISEASE

OBJECTIVE:

To present a tape study with video documentation to support the impressive, effective reversal of severe psychotic behavior with treatment protocols for Lyme disease.

METHODS:

The patient was diagnosed and treated in a Lyme specialized practice. A 16 year old white male first presented to this Southeast Pennsylvania practice on 5/27/00 after what has been described as a severe, psychotic episode. Apparently the patient had been involved in the use of smoking marijuana in the past week and had a psychotic episode. He was taken to an emergency room where he was given antipsychotic drugs. The patient then had some type of a reaction when he was given Risperdal and a dystonic, psychotic reaction occurred. The father was told that the patient would be in this condition for the rest of his life, that there was less than 1% of the population who has reactions to these medications. Previously to this episode the patient had had a dramatic drop in grades in the fall and winter from being an honor student. His mother and father are of high intelligence. It was later confirmed that the father, age 53, and in questionable health, had multiple EM rashes down both his arms and was positive for Lyme disease. Mother, age 50, had history of depression and was on Serzone at the time. Also had multiple EM rashes up and down her legs. Laboratory studies are pending. On physical exam the patient had severe grimacing of the face, a sixth cranial nerve palsy, right greater than left, a positive EM rash in the occipital area. The patient was documented by video at this time. He was noncommunitive, grunting, making noises speaking under his breath and displaying severe psychotic behavior. Heart showed a systolic murmur 1/6. Lungs clear bilaterally. Abdomen soft, no organomegaly. Negative ECC, zero focal deficits except for the sixth cranial nerve palsy. The patient's EKG showed a nonspecific T wave abnormality with a short PR interval. The patient's laboratory results displayed a CDC positive IgG Western Blot for Lyme disease, an equivocal IgM Western Blot with Kd 39 band equivocal. The patient was cross-referenced to a separate lab and was positive by PCR DNA analysis for Lyme disease. The patient was negative for rickettsial diseases. The patient's CBC and SMAC were within normal limits. The patient was negative for rheumatoid arthritis, syphilis. The patient showed positive antibody protection for hepatitis B, negative for hepatitis A and C. UA pending. The patient showed abnormal T3 Uptake of 39, 35 mcg/dl being normal. At this time the patient was administered 2.5 million units of Bicillin LA IM. Within two hours of receiving the shot the parents report that the patient's psychotic behavior subsided and the patient began to cry asking the parents for help. By the next morning the patient's psychotic behavior had returned. At this time the patient was not wanting to take oral medications. Within three days another IM shot of Bicillin was administered with 2.4 million units, the patient started becoming more cooperative.

RESULTS:

After the patient received a third shot of Bicillin on April 2nd his psychotic behavior had reverted back to normal. The patient is now back in school on a modified program at present. Again, father at the time of admission at Horsham Mental Health Center, before patient was treated for Lyme disease, was told that his son may be in this institution for an indefinite period of time stating that he had some type of a drug reaction and tardive dyskinesia which occurs in 1% of patients that receive antipsychotic drugs and he may be in this state for the rest of his life. In this case the patient had completely reverted back to normal behavior and is off all psychotic meds.

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CONCLUSION:

Lyme disease presents in many unusual presentations. In this particular case the patient was from a highly endemic West Chester, Pennsylvania area in which the patient's neighbors were being treated for Lyme disease. As we well know through literature that Lyme disease can create psychiatric dysfunction, thus any cases of unusual behavioral changes should be ruled out for possible Lyme disease as an organic cause of acute psychotic behavior.

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LYME DOT ASSAY (LDA)

It is documented in the literature that *Borrelia burgdorferi*, the causative agent of Lyme disease is found in the bladder and its antigens are shed in the urine. Currently, there are two tests that can detect *B. burgdorferi* in the urine: Lyme Urine Antigen test (LUAT) and Lyme PCR test. Lyme Urine Antigen test is an antigen capture-inhibition ELISA test that detects *B. burgdorferi* specific proteins. Lyme PCR detects *B. burgdorferi* specific DNA. The limit of detection of the LUAT is 32 ng of *B. burgdorferi* antigens per ml. We describe here a Lyme Immuno-Dot Assay (LDA), that detects *B. burgdorferi* specific antigens in urine. In this study, the assay had specificity of 95% and the limit of detection was 12.5 ng of *B. burgdorferi* antigens per ml of urine. The LDA performed in conjunction with Lyme Multiplex PCR can increase the sensitivity of detection of *B. burgdorferi* in urine by ~50%. In addition we have developed a Reverse Western Blot (RWB) assay for detection of *B. burgdorferi* antigens in urine. The RWB assay is used for confirmation of LDA positive urine samples. Urines from a total of 121 different individuals, 55 with Lyme Disease like symptoms and 66 normal controls were tested by LDA, RWB and PCR.

Patients with Lyme Disease Like Symptoms A total of 55 patients' urine samples were tested. Of these 55 patients tested, 21 were positive by either LDA, PCR or RWB. The remaining 34 were negative by all 3 tests. Of these 21 positives, 6 were positive by PCR only and one was positive by RWB only. The remaining 14 were positive by LDA.

14 LDA Positive Patient Samples Of the 14 LDA positive patients, 10 were positive by either PCR or RWB or both. Of these 10, 4 were positive by both RWB and PCR, 4 were positive by PCR and 2 were positive by RWB.

Normal Population Samples Urines from 66 normal controls were tested. Of these, 65 were negative by all 3 tests. One patient's urine was positive by LDA, but negative by PCR and RWB.

Specificity Any sample that was positive by LDA, confirmed by either PCR, RWB or both, was considered a "true positive". Any sample that was negative by all 3 methods was considered a "true negative". Of the 118 individuals samples, 95 were considered "true negative". Five individuals, 4 patients with Lyme Disease like symptoms and one normal control were positive by LDA, but negative by either PCR or RWB. Therefore these were considered "false positives". Based on these data, the specificity of LDA in this study is 95%.

Sensitivity Of the 17 "true positives", 10 samples were positive by LDA. Based on the limited data, the sensitivity of the LDA is 60%. The number of "true positives" increased from 10 to 16 (88%) when Lyme Multiplex PCR was added.

Conclusion Thus, we suggest that LDA and Lyme Multiplex PCR be performed on all urine samples for maximum sensitivity for detecting *Borrelia* in urine.

Nick S. Harris, Hui Mien Chen, Hua Bai and Jyotsna S. Shah*, IGeneX Inc., Palo Alto, CA 94303.

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Ehrlichia PCR Panel Test – Human Granulocytic Ehrlichia (HGE) and Human Monocytic Ehrlichia (HME) PCR Test

Ehrlichiosis is an acute, sometimes fatal febrile syndrome. It is caused by an intracellular bacterium, Human Granulocytic Ehrlichia (HGE) or Human Monocytic Ehrlichia (HME). Current diagnostic methods include Indirect Immuno-fluorescence assays and PCR. We describe here a PCR method that is highly specific and sensitive for the detection of HGE and HME directly from serum. Our proprietary target selection procedure selectively purifies the DNA of interest directly from serum. The purified DNA of interest is then amplified with Ehrlichia specific primers, using Polymerase Chain Reaction (PCR). Any sample that is positive by the Ehrlichia screen assay is confirmed as either HGE or HME using specific primers. The limit of detection is between 1- 5 Ehrlichia per 0.1ml of serum sample. A total of 140 serum samples were tested, 128 from patients with Ehrlichiosis like symptoms and 12 from normal controls. In addition, 24 Ehrlichia positive DNAs were tested.

Ehrlichia Screen PCR

Of the 128 clinical samples tested, 10 were positive for Ehrlichia. The remaining 118 were negative. All 12 normal control sera were negative for Ehrlichia. Of the 24 DNAs tested, 22 were positive for Ehrlichia. The remaining two had no DNA.

HGE and HME PCR

All 10 Ehrlichia positive clinical samples, and 22 Ehrlichia positive DNAs were tested by HGE and HME PCR assays. In addition, PCR products from the 10 Ehrlichia positive clinical samples were sequenced. Seven clinical samples were positive by HGE PCR. These were confirmed as HGE by sequencing. The remaining 3 were neither HGE or HME. All the 10 Ehrlichia positive clinical samples were negative by HME PCR assay. Of the 22 Ehrlichia positive DNA samples, 12 were HGE and 10 were HME. The 12 HGE were positive by HGE PCR and the 10 HME were positive by HME PCR. A few of these too were confirmed by DNA sequencing.

Based on the data presented we conclude that the HGE and HME PCR assays are highly specific and can be used to detect Ehrlichia in serum samples.

Jyotsna S. Shah*, Grace Hsui, Suzanne Ward and Nick Harris, IGeneX Inc., 797 San Antonio Road, Palo Alto, CA 94303.

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Off-Season Lyme Disease

PREMISE: Lyme disease has long been assumed to be a disease of the Summer and early fall for both early and late Lyme disease during the time of peak tick exposure.

HYPOTHESIS: The incidence of Lyme disease must be extended to off-season periods in the calendar due to delays in clinical expression.
The seasonality of Lyme disease presentation was analyzed in a hyperendemic area. The season of onset was reviewed for all Lyme disease cases confirmed by a positive ELISA and IgG Western Blot in our surveillance database, a base containing more than 2,000 patients evaluated between June 1997 and November 2000. The seasonal variations of Lyme disease occurrence together with the outcome of treatment by season were also evaluated.

RESULTS: A total of 33 of the 103 (32%) patients presented during the winter and spring -- 30 with chronic Lyme disease, and 3 with an erythema migrans rash. 30 of 33 patients (89%) had a delay in clinical symptoms to winter and spring consistent with the hypothesis of delays in clinical expression. 6 of 33 patients (17%) presenting in off-season delayed bringing their symptoms to the attention of their physician. 6 of 33 patients (17%) were examined by a physician but treatment was delayed. The patients presenting off-season had the same success rate but required longer duration of treatment.

CONCLUSIONS: In an endemic or hyperendemic area, Lyme disease in off-season is a common clinical presentation. The high incidence of chronic Lyme disease during the winter and spring can be unfamiliar to patients and their clinicians and lead to unnecessary and detrimental delays in treatment. These results suggest that beneath the well-established acute Lyme disease presentation with erythema migrans rash lies an endemic all-season chronic Lyme disease problem.

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ASSOCIATION OF MOTOR NEURON DISEASE (MND) AND LYME DISEASE (LD). Liegner KB* & Sharahy T. Private practice, Armonk, NY. In the past ten years we have seen fourteen patients having evidence of LD and MND. Laboratory evidence for LD in these patients varied from the presence of a few highly specific bands on IgG and/or IgM Lyme Western blot to fully diagnostic IgG blots with positive Lyme ELISAs in some, and/or detection of DNA of the Lyme organism in bodily fluids by PCR at a laboratory participating in and satisfying the College of American Pathology's Proficiency Testing Program for Lyme PCR. Motor neuron involvement, often termed "atypical", was diagnosed by a neurologist in each case. In two of the patients application of oral and/or intravenous antibiotic therapy appeared to resolve and avert progression of the illness. For the remainder, antibiotic therapy did not seem to ameliorate the MND, which was progressive and ended in death for three of the fourteen subjects at last known follow-up. Causal relationship between borrelial infection and occurrence of MND in these individuals is uncertain. Association of these two illnesses in fourteen patients within one practice specializing in LD suggests that epidemiologic study by public health authorities of the incidence of MND in endemic areas before and following the burgeoning of LD in the population would be of value. Linkage of MND and borrelial infection, if *bona fide*, could implicate one specific infectious agent in causing some cases of MND and lend insight into etiopathogenesis of MND in general. If a causal relation exists in LD-associated MND injury might be due to immunopathogenic mechanisms, neurotoxin based injury to motor neurons, or noxious effects due to direct borrelial invasion of anterior horn cells. Establishment of a NINDS study section for individuals evidencing MND and LD should be considered as a resource and referral option for clinicians encountering these patients. Such a study section could further understanding of a possible borrelial etiopathogenesis in MND. This, in turn, may lead to advances in diagnosis, prevention and treatment of LD-associated MND as well as MND due to other etiologies.

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