Seronegativity in Lyme borreliosis and Other Spirochetal Infections

16 September 2003

"If false results are to be feared, it is the false negative result which holds the greatest peril for the patient."

Gestational Lyme borreliosis. Implications for the fetus. MacDonald AB. *Rheum Dis Clin North Am*, 15(4):657-77. 1989.

Borrelia burgdorferi

	Author	*Year*	*Title*	*Journal*
1.	**Dejmkova H; Hulinska D; Tegzova D; Pavelka K; Gatterova J; Vavrik P.**	2002	**Seronegative Lyme arthritis caused by Borrelia garinii.**	Clinical Rheumatology, 21(4):330-4

[From the abstract:] "A case of a female patient suffering from Lyme arthritis (LA) without elevated antibody levels to Borrelia burgdorferi sensu lato is reported. Seronegative Lyme arthritis was diagnosed based on the classic clinical manifestations and DNA-detected Borrelia garinii in blood and synovial fluid of the patient, after all other possible causes of the disease had been ruled out. The disease was resistant to the first treatment with antibacterial agents. Six months after the therapy, arthritis still persisted and DNA of Borrelia garinii was repeatedly detected in the synovial fluid and the tissue of the patient. At the same time, antigens or parts of spirochaetes were detected by electron microscopy in the synovial fluid, the tissue and the blood of the patient. The patient was then repeatedly treated by antibiotics and synovectomy has been performed."

2.	**Tylewska-Wierzbanowska S; Chmielewski T;**	2002	**Limiation of serologic testing for Lyme borreliosis: evaluation of ELISA and western blot in comparison with PCR and culture methods.**	Wien Klin Wochenschr, 114(13-14):601-5

[From the abstract:] "No correlation was found between levels of specific B. burgdorferi antibodies detected with a recombinant antigen ELISA and the number of protein fractions developed with these antibodies by immunoblot. Moreover, Lyme borreliosis patients who have live spirochetes in body fluids have low or negative levels of borrelial antibodies in their sera. This indicates that an efficient diagnosis of Lyme borreliosis has to be based on a combination of various techniques such as serology, PCR and culture, not solely on serology." [Testing was performed on samples from 90 patients.]

3.	**Breier F; Khanakah G; Stanek G; Kunz G; Aberer E; Schmidt B; Tappeiner G.**	2001	**Isolation and polymerase chain reaction typing of Borrelia afzelii from a skin lesion in a seronegative patient with generalized ulcerating bullous lichen sclerosus et atrophicus.**	Br J Dermatol, 144(2):387-392

[From the abstract:] "Spirochaetes were isolated from skin cultures obtained from enlarging LSA lesions. These spirochaetes were identified as Borrelia afzelii by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and polymerase chain reaction (PCR) analyses. However, serology for B. burgdorferi sensu lato was repeatedly negative."

4.	**Brunner M.**	2001	**New method for detection of Borrelia burgdorferi antigen complexed to antibody in seronegative Lyme disease.**	J Immunol Methods, 249(1-2):185-190

[From the abstract:] "...serologic tests for early Lyme disease can be falsely negative due to lack of sensitivity of ELISAs and Western blots. Most routine antibody tests are designed to detect free antibodies, and in early, active disease, circulating antibodies may not be free in serum but sequestered in complexes with the antigens which originally triggered their production. This difficulty may be overcome by first isolating immune complexes (IC) from the serum and using this fraction for testing. Free Borrelia-specific antibodies can then be liberated from the immune complexes which may enhance test sensitivity in patients with active disease. We developed a technique that captures the antibody component of IC on immunobeads, and subsequently releases the antigen component of IC. Immunoblotting with monoclonal antibody detected at least one antigen to be OspA, thus definitively demonstrating a Borrelia-specific antigen in circulating IC in early Lyme disease. This test is also useful in demonstrating Bb antigen in otherwise seronegative Lyme disease patients."

	Author	Year	Title	Journal
5.	**Wang P; Hilton E.**	2001	**Contribution of HLA alleles in the regulation of antibody production in Lyme disease.**	Front Biosci, 6:B10-B16

"Of eighteen seronegative LD patients, 14 were OspA PCR positive on mononuclear cells and 5 were positive on CSF. ...The presence of certain HLA alleles with seronegativity to disease has been reported in malaria (10), HIV (16,17), rheumatoid arthritis (RA) (18) and spondylarthropathies (SpA) (19). ...Our results provide evidence of a correlation between certain HLA genotypes and the ability to mount an antibody response to Bb. In this study, 9 of 22 (40.9%) seropositive LD patients and only 1 out of 18 (5.6%) seronegative LD patients had HLA-DR7 alleles. ...

Our study provides evidence that HLA alleles are involved in antibody responsiveness or non-responsiveness to Bb infection. A low frequency of HLA-DR7 alleles and HLA-DR6 alleles and a high frequency of HLA-DR1 alleles may contribute to non-responsiveness of antibody production in LD patients. Thus, genetic predisposition may be a critical factor in the regulation of the host immune response and the diagnosis and prognosis of Lyme disease."

6.	**Grignolo MC; Buffrini L; Monteforte P; Rovetta G.**	2001	**Reliability of a polymerase chain reaction (PCR) technique in the diagnosis of Lyme borreliosis.**	Minerva Med, 92(1):29-33

[From the abstract:] "50% of the PCR positive results, obtained with serum and cerebrospinal fluid samples corresponded to patients who were true positives at clinical examination but negatives at serologic tests. 62.5% of urine samples positive results belonged to tp patients who had negative serologic and serum PCR RESULTS. CONCLUSIONS: The obtained results suggested a good reliability of positive results obtained with the PCR technique used in this study and allowed the false negatives of serologic tests to be detected, more specifically when urine samples were used."

7.	**Klempner MS; Schmid CH; Hu L; Steere AC; Johnson G; McCloud B; Weinstein A.**	2001	**Intralaboratory reliability of serologic and urine testing for Lyme disease.**	American Journal of Medicine, 110(3):217-19

"In the 21 patients with Lyme disease, the results of the initial western blot analysis were positive in 14 cases and negative in 7. ... Repeat testing of the 7 seronegative samples showed fewer than 5 reactive bands in all samples."

8.	**Honegr K; Hulinska D; Dostal V; Gebousky P; Hankova E; et al.**	2001	**[Persistence of Borrelia burgdorferi sensu lato in patients with Lyme borreliosis].**	Epidemiol Mikrobiol Imunol, 50(1):10-6

[From the abstract:] "In 18 patients with Lyme borreliosis the authors proved the persistence of Borrelia burgdorferi sensu lato by detection of the causal agent by immune electron microscopy or of its DNA by PCR in plasma or cerebrospinal fluid after an interval of 4-68 months. ...Examination of antibodies by the ELISA method was negative in 7 of 18 patients during the first examination and in 12 of 18 during the second examination. In all negative examinations the specific antibodies were assessed by the Western blot or ELISA method after liberation from the immunocomplexes."

9.	**Paul A.**	2001	**[Arthritis, headache, facial paralysis. Despite negative laboratory tests Borrelia can still be the cause.]**	MMW Fortschr Med, 143(6):17

10.	**Pleyer U; Priem S; Bergmann L; Burmester G; Hartmann C; Krause A.**	2001	**Detection of Borrelia burgdorferi DNA in urine of patients with ocular Lyme borreliosis.**	Br J Ophthalmol, 85(5):552-5

[From the abstract:] "RESULTS: Only four of six uveitis patients suspected for Lyme borreliosis were ELISA positive, while all six subjects showed a positive western blot. B burgdorferi PCR was positive in all of these six patients. Whereas two of the 30 controls had a positive Lyme serology, B burgdorferi DNA was not detectable by PCR in any sample from these patients. CONCLUSIONS: PCR for the detection of B burgdorferi DNA in urine of uveitis patients is a valuable tool to support the diagnosis of ocular Lyme borreliosis. Moreover, these patients often show a weak humoral immune response which may more sensitively be detected by immunoblotting."

11.	**Eldoen G; Vik IS; Vik E; Midgard R.**	2001	**[Lyme neuroborreliosis in More and Romsdal].**	Tidsskrift for Den Norske Laegeforening, 121(17):2008-11.

[From the abstract:] "Fourteen of 25 (56%) patients had positive Borrelia burgdorferi-IgM and IgG titres in cerebrospinal fluid despite negative tests in serum."

Author	*Year*	*Title*	*Journal*
12. **Brunner M; Sigal LH.**	2000	**Immune complexes from serum of patients with Lyme disease contain Borrelia burgdorferi antigen and antigen-specific antibodies: potential use for improved testing.**	Journal of Infectious Diseases, 182(2):534-9

[From the abstract:] "We report sequestration of specific IgM anti-Borrelia burgdorferi (Bb) and Bb antigens within immune complexes (ICs) isolated from serum of patients with Lyme disease (LD). ...Immunoblot demonstrated that ICs contained antibodies against specific Bb proteins, whereas reactivity was absent or significantly lessened in unprocessed serum."

13. **Kaiser R.** 2000 **False-negative serology in patients with neuroborreliosis and the value of employing of different borrelial strains in serological assays.** J Med Microbiol, 49(10):911-5.

[Abstract:] "The risk of obtaining false-negative results in serological assays in serum and CSF specimens with only one strain of Borrelia burgdorferi sensu lato as antigen was investigated in 79 patients with neuroborreliosis with specimens obtained at initial presentation. Serum antibodies were assessed by immunoblotting; the criteria of Hauser et al. were used to evaluate the test. The intrathecal synthesis of borrelial-specific IgM and IgG antibodies was examined by enzyme immunoassay (EIA). Strains of B. burgdorferi sensu stricto (BbZ160), B. garinii (Bbii50) and B. afzelii (PKO) served as sources of antigen in both assays. All patients produced either a positive IgM or IgG test in serum with at least one strain of B. burgdorferi sensu lato. Reactivity of IgM or IgG antibodies, or both, with antigens of all three strains was demonstrated in 67 (85%) of 79 sera. The correlation of results of immunoblotting with different strains was significantly better for IgG (85%) than for IgM antibodies (54%). The variability of positive IgM reactions in 18 specimens was mainly due to the fact that the antibodies were directed to the relevantvariable outer-surface protein C (p23). Intrathecal synthesis of IgG antibodies was demonstrated in 58 patients (81%) of 72 and of IgM antibodies in 25 of 58 patients. No patient had isolated intrathecal synthesis of IgM antibodies. The majority of CSF samples (56 of 58) were assessed as IgG antibody-positive, independent of the borrelial strain used as antigen in EIA, whereas only 10 of 25 IgM antibody-positive CSF specimens reacted with all three strains. All patients in the study had intrathecal antibody synthesis demonstrable at 6-week follow-up. From this study it is concluded that there is a small, but real, risk of false-negative serological findings at the time of initial clinical presentation in patients with typical symptoms of neuroborreliosis. In these patients a negative serological result with one strain should prompt the repetition of the test with other strains of B. burgdorferi sensu lato."

14. **Kmety E.** 2000 **[Dynamics of antibodies in Borrelia burgdorferi sensu lato infections.]** Bratisl Lek Listy, 101(1):5-7

[From the abstract:] "...During 1994-1998 at least two serum samples were submitted for serological testing from more than 1200 patients. An immunofluorescence test was performed paralelly [sic] with two pools of antigen (B. bg.s.s. + B. afzelii, and two serological different strains of B. garinii, all of local origin). In 92-96% of patients no change of antibody level was found in repeated tests, about 20% of them being negative (< 1:512).

...Only in 9 cases a rise of the titer appeared during 3 weeks after the first negative sample, at contrary in 7 cases no rise of the titer was seen in that time. 2 patients were still after 1 month, 3 after 3 months and 1 even after 7 months (patient with a positive CSF culture) serologically negative."

15. **Wilke M; Eiffert H; Christen HJ; Hanefeld F.** 2000 **Primarily chronic and cerebrovascular course of Lyme neuroborreliosis: case reports and literature review.** Arch Dis Child, 83(1):67-71.

"In this context, even the complete absence of specific antibodies has been observed; in a girl diagnosed as having focal vasculitis through CNS biopsy, the presence of B burgdorferi in CSF was confirmed by polymerase chain reaction. No specific antibodies were detectable. In three other children, B. burgdorferi could be cultured from CSF in the absence of specific antibodies in CSF or blood."

16. **Sheets JT; Rossi CA; Kearney BJ; Moore GE.** 2000 **Evaluation of a commercial enzyme-linked immunosorbent assay for detection of Borrelia burgdorferi exposure in dogs.** J Am Vet Med Assoc, 216(9):1418-22

"The commercial ELISA kit evaluated in this study appeared to lack adequate sensitivity for detecting all potential cases of borreliosis in dogs."

	Author	*Year*	*Title*	*Journal*
17.	**Wang P; Gartenhaus R; Sood SK; DeVoti J; Singer C; et al.**	2000	**Detection of Borrelia DNA in circulating monocytes as evidence of persistent Lyme disease.**	J Spirochetal and Tick-borne Diseases, 7(1):16-19
			[Abstract:] "We report the detection of Borrelia burgdorferi DNA in circulating monocytes in a 31-year-old female who presented with a flu-like syndrome followed by neurological abnormalities after a trip to Southampton, Long Island, New York. ELISA and Western blot were negative. Lymphocyte proliferation assay to Borrelia burgdorferi was positive. Borrelia burgdorferi DNA was detected in circulating monocytes using a nested polymerase chain reaction (PCR). Treatment with parenteral ceftriaxone resulted in clinical improvement and repeat PCR on monocytes was negative. The use of detecting DNA by PCR from circulating monocytes may be useful in evaluating seronegative patients with a high suspicion of Lyme disease."	
18.	**Brown SL; Hansen SL; Langone JJ.**	1999	**Role of serology in the diagnosis of Lyme disease. (FDA Medical Bulletin)**	JAMA, 282(1): 62-65
			"*The Food and Drug Administration (FDA) is concerned about the potential for misdiagnosis of Lyme disease based on the results of commonly marketed tests for detecting antibodies to Borrelia burgdorferi, the organism that causes Lyme disease. It is important that clinicians understand that a positive test result does not necessarily indicate current infection with B. burgdorferi, and a patient with active Lyme disease may have a negative test result.*	
			The tests should be used only to support a clinical diagnosis of Lyme disease and should never be the primary basis for making diagnostic or treatment decisions."	
19.	**Bertrand E; Szpak GM; Pilkowska E; Habib N; et al.**	1999	**Central nervous system infection caused by Borrelia burgdorferi. Clinico-pathological correlation of three post-mortem cases.**	Folia Neuropathol, 37(1):43-51
			"Case 1: ...Specific borrelia IgM and IgG value in serum and CSF were normal (<250). However, on microscopical examination the spirochete B. burgdorferi was demonstrated in serum and CSF. The bacteria were cultured both from blood and from CSF, in CSF they were also identified by PCR."	
20.	**Mikkila H, Karma A, Viljanen M, Seppala I.**	1999	**[The laboratory diagnosis of ocular Lyme borreliosis.]**	Graefes Arch Clin Exp Ophthalmol, 237(3):225-30
			"Seven patients, including two with negative ELISA, had a positive immunoblot. Seven of the 13 patients in whom PCR was examined during clinically active disease had a positive PCR result. Immunoblot analysis gave a negative result from the sera of five PCR-positive patients. CONCLUSIONS: For efficient diagnosis of ocular Lyme borreliosis, immunoblot analysis and PCR should be used in addition to ELISA."	
21.	**Oksi J; Marjamaki M; Nikoskelainen J; Viljanen MK.**	1999	**Borrelia burgdorferi detected by culture and PCR in clinical relapse of disseminated Lyme borreliosis.**	Annals of Medicine, 31(3):225-32
			"Three of the 13 patients had only IgM antibodies against B. burgdorferi, and one culture-positive patient was seronegative despite the disseminated stage of the disease. The reason for the lack of IgG antibodies, or of both IgM and IgG antibodies, was not restriction of the infection to privileged sites, as all these patients had a multiorgan disease. We have previously shown that patients with late LB with live spirochetes or borrelial DNA in their body fluids may have low or negative serum borrelia antibody levels."	
22.	**Hudson BJ; Stewart M; Lennox VA; et al.**	1998	**Culture-positive Lyme borreliosis.**	Med J Aust, 168(10):500-2
			[From the abstract:] "We report a case of Lyme borreliosis. Culture of skin biopsy was positive for Borrelia garinii, despite repeated prior treatment with antibiotics.'	
			"The results of conventional serological and histopathological tests were negative, despite an illness duration of at least two years."	

	Author	*Year*	*Title*	*Journal*
23.	**McCaulley, Mark E., M.D.**	1998	**Guidelines for the clinical diagnosis of Lyme disease.**	Annals of Internal Medicine, 129(5): 422-423

[Letter to the Editor:] "The position paper on laboratory diagnosis of Lyme disease is based on a widely accepted paradigm that is inconsistent with a growing body of medical literature. According to this paradigm, cases of Lyme disease are overwhelmingly seropositive and are unlikely to be associated with persistent symptoms after presumed adequate therapy. In addition, any patients remaining persistently symptomatic are presumed to no longer have Lyme disease at all but rather to have such conditions as fibromyalgia, depression, or the chronic fatigue syndrome and, as a result, to be unlikely to respond to additional antibiotic therapy. Such presumptions are inconsistent with an increasing number of reports.

A 1994 article reports the increased frequency of multiple symptoms in previously treated patients with Lyme disease compared with controls. Antibodies on ELISA were found in less than half of the patients with Lyme disease. Re-treatment was associated with improvement in half of re-treated patients. Had the guidelines been followed in a clinical evaluation of these or similar patients, Lyme disease would have been diagnosed in few of them.

In a 1996 report, Borrelia burgdorferi plasmid DNA was detectable by polymerase chain reaction assay only in a subset of patients with Lyme disease who were seronegative. Many case reports have described patients with Lyme disease who remain antigen positive and symptomatic despite intensive antibiotic treatment.

I suggest the acceptance of a new paradigm that incorporates the above information. Physicians involved in the treatment of Lyme disease should consider that 1) Patients with Lyme disease, especially those in late stages of the disease, are frequently seronegative; 2) the persistence of symptoms, which may be vague, is common and may respond to additional antibiotic therapy; and 3) there is much to be learned about the optimal treatment of Lyme disease at any stage."

24.	**Petrovic M; Vogelaers D; Van Renterghem L; Carton D; De Reuck J; Afschrift M.**	1998	**Lyme borreliosis - a review of the late stages and treatment of four cases.**	Acta Clinica Belgica, 53(3):178-83

[From the abstract:] "Difficulties in diagnosis of late stages of Lyme disease include low sensitivity of serological testing and late inclusion of Lyme disease in the differential diagnosis. Longer treatment modalities may have to be considered in order to improve clinical outcome of late disease stages...The different clinical cases illustrate several aspects of late borreliosis: false negative serology due to narrow antigen composition of the used ELISA format, the need for prolonged antibiotic treatment in chronic or recurrent forms and typical presentations of late Lyme disease, such as lymphocytic meningo-encephalitis and polyradiculoneuritis."

25.	**American Academy of Neurology 49th Annual Meeting April 12-19.**	1997	**Lyme encephalopathy may surface despite antibiotic treatment.**	http://www.medscape.com/CPG/ClinReviews/1997/v07.n06/c0706.cnu/c0706.cnu.html#Lyme

"Of the 8 patients with CNS infection, only 2 were seropositive on both the ELISA and Western blot tests. Four had indeterminate ELISA results and a negative Western blot, and 2 had negative results on both the ELISA and the Western blot. Neither of the 2 seropositive patients had received antibiotics during the first month of infection for early localized or disseminated disease," said the Boston researchers. Of the 6 seronegative patients with CNS infection, however, 5 (84%) had received a recommended course of oral or intravenous antibiotics during the first month of infection.'"

26.	**Branigan P; Rao J; Rao J; Gerard H; Hudson A; Williams W; Arayssi T; Pando J; Bayer M; Rothfuss S; Clayburne G; Sieck M; Schumacher HR.**	1997	**PCR evidence for Borrelia burgdorferi DNA in synovium in absence of positive serology.**	American College of Rheumatology, Vol 40(9), Suppl:S270

"PCR evidence for Borrelia has been identified in synovial biopsies of patients with clinical pictures that had not initially suggested Lyme disease. All 6 PCR-positive] patients were negative for antibodies to Borrelia and some were PCR positive in synovium despite previous treatment with antibiotics."

	Author	*Year*	*Title*	*Journal*
27.	**Donta ST.**	1997	**Tetracycline therapy for chronic Lyme disease.**	Clin Infect Dis, Jul;25 Suppl 1:S52-6
			"Treatment outcomes for seronegative patients (20% of all patients) were similar to those for seropositive patients. Western immunoblotting showed reactions to one or more Borrelia burgdorferi-specific proteins for 65% of the patients for whom enzyme-linked immunosorbent assays were negative."	
28.	**Hauser U; Wilske B.**	1997	**Enzyme-linked immunosorbent assays with recombinant internal flagellin fragments derived from different species of Borrelia burgdorferi sensu lato for the serodiagnosis of of Lyme.**	Medical Microbiology & Immunology. 186(2-3):145-51
			[From the abstract:] "The serodiagnosis of early Lyme neuroborreliosis is hampered by false negative results and one of the reasons could be the heterogeneity of strains of Borrelia burgdorferi sensu lato."	
29.	**Pradella SP; Krause A; Muller A.**	1997	**Acute Borrelia infection. Unilateral papillitis as isolated clinical manifestation.**	Ophthalmologe, Aug;94(8):591-4
			[From the abstract:] "Seronegative values in subjects strongly suspected of having Lyme disease do not necessarily exclude the diagnosis of Lyme disease."	
30.	**Schumacher HR.**	1997	**PCR evidence for Borrelia burgdorferi DNA in synovium in absence of positive serology.**	Abstract ACR 61st National Scientific Meeting November 8-12
31.	**Aberer E; Kersten A; Klade H; Poitschek C; Jurecka W.**	1996	**Heterogeneity of Borrelia burgdorferi in the skin.**	American Journal of Dermatopathology, 18(6):571-9
			"Neuralgias arising 6 months after ECM in spite of antibiotic therapy were evident in a seronegative patient who showed perineural rod-like borrelia structures."	
			"The morphological forms of borreliae seen in biopsies were correlated with clinical findings. Seropositive patients showed clumped and agglutinated borreliae in tissue, whereas seronegative patients exhibited borreliae colony formation (n=2). ...the behavior of borreliae within collagen fibers is strongly influenced by immune recognition by the patient. Borrelia may escape immune surveillance by colony formation and masking within collagen, resulting in seronegativity."	
32.	**Breier P; Klade H; Stanek G; Poitschek C; Kirnbauer R; Dorda W; Aberer E.**	1996	**Lymphoproliferative responses to Borrelia burgdorferi in circumscribed scleroderma.**	Br J Dermatol, 134(2):285-91
			"These findings show that the pattern of Bb-specific immune responses is more complex than previously thought, and underscore the importance of lymphocyte function assays in evaluating the diagnosis of potential Bb infection in seronegative patients."	

	Author	Year	Title	Journal
33.	**Huppertz HI; Mosbauer S; Busch DH; Karch H.**	1996	**Lymphoproliferative responses to Borrelia burgdorferi in the diagnosis of Lyme arthritis in children and adolescents.**	Eur J Pediatr, 155(4):297-302
			"In one patient with seronegative LA [Lyme arthritis] specific lymphocyte proliferation and polymerase chain reaction for borrelial fla sequences in urine were positive."	
34.	**Luft BJ.**	1996	**Chronic Lyme disease: an evolving syndrome.**	9th Annual International Scientific Conference on Lyme Disease & Other Tick-Borne Disorders, Boston, MA, April 19-20
			[From the abstract:] "In the case of the ticks, environmental factors such as temperature, humidity and source of blood meal may alter the major outer surface proteins (Osp) of the spirochete within the tick vector. ...Humans with chronic arthritis are more likely to show an immune response to Osp A." *[Seronegativity:] "Chronic Lyme disease patients may be seropositive or seronegative with or without a documented history of Lyme disease."* *[Diagnosis:] "Since Lyme disease is a clinical diagnosis, research must continue to improve diagnostic assays using recombinant proteins which are more sensitive and specific than the whole organism sonicate used for both ELISA and Western blots."*	
35.	**Luft BJ; Dattwyler RJ; Johnson RC; Luger SW; Bosler EM; Rahn DW; et al.**	1996	**Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial.**	Annals of Internal Medicine, 124(9):785-91
			"Fifty-seven percent of patients who had relapse were seronegative at the time of relapse."	
36.	**Mouritsen CL; Wittwer CT; Litwin CM; Yang L; Weis JJ; Martins TB; Jaskowski TD; Hill HR.**	1996	**Polymerase chain reaction detection of Lyme disease: correlation with clinical manifestations and serologic responses.**	American Journal of Clinical Pathology. 105(5):647-54
			[From the abstract:] "...nine serum samples and one synovial fluid from patients with definite clinical features of Lyme disease were found to be negative by EIA and Western blot analysis for IgG and IgM antibody, but contained B burgdorferi DNA, as detected by PCR. Polymerase chain reaction analysis of serum and synovial fluid may be of significant diagnostic value in Lyme disease, especially in the absence of a serologic response in early, partially treated and seronegative chronic disease....This is the first study to report an association between PCR positivity and the absence of a serologic response to Lyme borreliosis."	
37.	**Mursic VP; Wanner G; Reinhardt S; Wilske B; et al.**	1996	**Formation and cultivation of Borrelia burgdorferi spheroplast L-form variants.**	Infection, 24(3):218-26
			This study investigated In vitro morphological variants of B. burgdorferi, in an effort to explain the clinical persistence of active Lyme borreliosis despite antibiotic therapy. The authors suggest that these atypical forms may allow Borrelia to survive antibiotic treatment. *"Penicillin G was the most effective inducer of SL-forms [spheroplast-L-forms). The reversion of this form to the helical parental forms was mostly achieved by cultivation of isolated SL-colonies in penicillin G-free medium. The atypical forms isolated from patients treated with antibiotics show similar features. The same effect is probably obtained with all other ß-lactam antibiotics."* *"With regard to the polyphasic course of Lyme borreliosis, these forms without cell walls can be a possible reason why Borrelia survive in the organism for a long time (probably with all beta-lactam antibiotics) [corrected] and the cell-wall-dependent antibody titers disappear and emerge after reversion."*	
38.	**Preac Mursic V; Marget W; Busch U; Pleterski Rigler D; Hagl S.**	1996	**Kill kinetics of Borrelia burgdorferi and bacterial findings in relation to the treatment of Lyme borreliosis.**	Infection, 24(1):9-16
			"The patients had clinical disease with or without diagnostic antibody titers to B. burgdorferi."	

	Author	Year	Title	Journal
39.	**Pachner A.**	1995	**Early disseminated Lyme disease.**	Am J Med, 98 (suppl 4A):4A-30S-51S – Discussion.

"The correlation between a positive Western blot and Lyme arthritis is probably the best of almost any Western blot and any Lyme disease manifestation. With neurologic disease, I have had a lot of patients who don't have a positive Western blot; they just have not developed a peripheral antibody response, for whatever reason."

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40. **Coyle PK; Schutzer SE; Deng Z; Krupp LB; Belman MD; Benach JL; Luft BJ.** 1995 **Detection of Borrelia burgdorferi-specific antigen in antibody negative cerebrospinal fluid in neurologic Lyme disease.** Neurology, 45:2010-2014

[From the abstract:] " RESULTS: Of the 35 of 83 (42%) patients who were positive for OspA antigen in their CSF, 15 (43%) were antigen positive despite being antibody-negative in CSF. Seven of these 15 (47%) had otherwise normal routine CSF analyses. Six of these 15 (40%) patients met strict CDC surveillance criteria for Lyme disease: four (27%) patients had seroconversion coincident with new neurologic problems; and three (20%) with characteristic syndromes for Lyme disease were seronegative, but had complexed antibody to B. burgdorferi. The final two patients (13%) were seropositive and had unexplained neurologic problems not characteristic of Lyme disease. CONCLUSIONS: B. burgdorferi antigen can be detected in CSF that is otherwise normal by conventional methodology, and can be present without positive CSF antibody. Since CSF antigen implies intrathecal seeding of the infection, the diagnosis of neurologic infection by B. burgdorferi should not be excluded solely on the basis of normal routine CSF or negative CSF antibody analyses."

[From the article:] "Prompt and precise diagnosis is difficult because basic microbiologic tests such as culture and staining have not been useful, on a broad scale, to document the presence of the spirochete in a body fluid. Instead, detection of specific antibodies to B burgdorferi in blood and CSF is commonly used to support or refute a clinical suspicion of infection. Many of the commercially available assays have been plagued by lack of sensitivity, specificity, and reproducibility. Furthermore, the absence of free antibodies to B burgdorferi components has been documented in well-characterized erythema-migrans-positive cases of Lyme disease, including those with prominent neurologic involvement."

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41. **Karma A; Seppala I; Mikkila H; Kaakkola S; Viljanen M; Tarkkanen A.** 1995 **Diagnosis and clinical characteristics of ocular Lyme borreliosis.** American Journal of Ophthalmology, 119(2):127-35

[From the abstract:] "Results of ELISA disclosed that five patients [out of ten] were seropositive, two patients showed borderline reactivity, and three patients were seronegative. Four of the five patients with borderline or negative results by ELISA had a positive result by western blot analysis. ... CONCLUSIONS: Late-phase ocular Lyme borreliosis is probably underdiagnosed because of weak seropositivity or seronegativity in ELISA assays."

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42. **Lawrence C; Lipton RB; Lowy FD; Coyle PK.** 1995 **Seronegative chronic relapsing neuroborreliosis.** European Neurology, 35(2):113-7

[From the abstract:] This article reports a Lyme disease patient "who experienced repeated neurologic relapses despite aggressive antibiotic therapy." The patient was seronegative. "Although the patient never had detectable free antibodies to B. burgdorferi in serum or spinal fluid, the CSF was positive on multiple occasions for complexed anti-B. burgdorferi antibodies, B. burgdorferi nucleic acids and free antigen."

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43. **Millner M.** 1995 **Neurologic manifestations of Lyme borreliosis in children.** Wiener Medizinische Wochenschrift,145(7-8):178-82

"Our own observations in children which suffered from an acute neuroborreliosis (NB) showed the following:... Indeed, there is a seronegative NB also in children."

	Author	*Year*	*Title*	*Journal*
44.	**Oksi J; Uksila J; Marjamaki M; Nikoskelainen J; Viljanen MK.**	1995	**Antibodies against whole sonicated Borrelia burgdorferi spirochetes, 41-Kilodalton flagellin, and P39 protein in patients with PCR- or culture-proven late Lyme borreliosis.**	Journal of Clinical Microbiology, 33(9):2260-4
			[From the abstract:] "These results show that antibodies to B. burgdorferi may be present in low levels or even absent in patients with culture- or PCR-proven late LB [Lyme borreliosis]. Therefore, in addition to serological testing, the use of PCR and cultivation is recommended in the diagnosis of LB."	
45.	**Skripnikova IA; Anan'eva LP; Barskova VG; Ushakova MA.**	1995	**The humoral immunological response of patients with Lyme disease.**	Ter Arkh, 67(11):53-6
			"Both acute and chronic borreliosis can be seropositive or seronegative."	
46.	**Schubert HD; Greenebaum E; Neu HC.**	1994	**Cytologically proven seronegative Lyme choroiditis and vitritis.**	Retina, 14(1):39-42
			[From the abstract:] "RESULTS: Intravitreal spirochetes consistent with Borrelia burgdorferi were found in this seronegative patient. CONCLUSION: Vitreous specimens of patients with choroiditis and vitritis of unknown cause should be examined cytologically, particularly when serologic results do not corroborate the clinical findings of Lyme disease."	
47.	**Sigal LH.**	1994	**The polymerase chain reaction assay for Borrelia burgdorferi in the diagnosis of Lyme disease.**	Annals of Internal Medicine, 120(6):520-521
			"Polymerase chain reaction may be more sensitive than antibody detection techniques in human Lyme neuroborreliosis [17,19] and the murine experimental model [22] and clearly is more sensitive than current culture techniques. Our experience suggests that a few patients may be positive by PCR despite negative immunologic assay results in inflammatory fluid and blood (Sigal LH and Liebling M. Unpublished observation)."	
48.	**Bojic I; Mijuskovic P; Dokic M; Nozic D; Lako B; et al.**	1993	**Clinical characteristics of Lyme disease.**	Vojnosanit Pregl, 50(4):359-64
			[From the abstract:] "Clinical characteristics of Lyme disease were analysed in 22 patients. Erythema migrans was found in 20 (91%), arthralgia in 18 (81%), neuralgia in 8 (36%), encephalitis in 3 (13%), carditis in 2 (9%) and arthritis in 2 (9%) patients. The positive antibody titer was found in 14 (63%) patients.	
49.	**Coyle PK.**	1993	**Antigen detection and cerebrospinal fluid studies.**	In "Lyme Disease," ed. P. Coyle, p.143
			"...spirochetes show a peculiar feature compared to other bacterial neurologic infections: the organisms can be present in CSF without inducing inflammatory changes. This is well-documented for neurosyphilis, leptospirosis, and relapsing fever, and appears to be occasionally true for Lyme disease as well. In Europe, B. burgdorferi has been cultured from otherwise normal CSF."	
50.	**Häupl T; Hahn G; Rittig M; Krause A; Schoerner C; Schonherr U; et al.**	1993	**Persistence of Borrelia burgdorferi in ligamentous tissue from a patient with chronic Lyme borreliosis.**	Arthritis & Rheumatism, 36(11):1621-6
			[From the abstract:] "The initially significant immune system activation was followed by a loss of the specific humoral immune response and a decrease in the cellular immune response to B burgdorferi over the course of the disease." [From the article:] "Interestingly, the cellular immune responses were also directed against the surface protein OspA during each recurrence of clinical symptoms, even though anti-OspA antibodies were not detectable by immunoblot."	

	Author	*Year*	*Title*	*Journal*
51.	**Hulinska D; Krausova M; Janovska D; et al.**	1993	**Electron microscopy and the polymerase chain reaction of spirochetes from the blood of patients with Lyme disease.**	Central European Journal of Public Health. 1(2):81-5
		[From the abstract:] "Results of studies using direct antigen detection suggest that seronegative Lyme borreliosis is not rare and support the hypothesis that Borrelia antigens can persist in humans."		
52.	**Kazakoff MA; Sinusas K; Macchia C.**	1993	**Liver function test abnormalities in early Lyme disease.**	Arch Fam Med, 2(4):409-13
		[From the abstract:] "PATIENTS: Thirty-seven female and 36 male patients with erythema migrans who had not yet been treated with antimicrobial agents. ... Only seven patients (9%) had a positive titer in response to the enzyme-linked immunosorbent assay for Lyme disease."		
53.	**Liegner KB; Shapiro JR; Ramsay D; Halperin AJ; Hogrefe W; Kong L.**	1993	**Recurrent erythema migrans despite extended antibiotic treatment with minocycline in a patient with persisting Borrelia burgdorferi infection.**	Journal of the American Academy of Dermatology, 28(2 Pt 2):312-4
		[Abstract:] "Erythema migrans recurred in a patient 6 months after a course of treatment with minocycline for Lyme disease. Polymerase chain reaction on heparinized peripheral blood at that time demonstrated the presence of Borrelia burgdorferi-specific DNA. The patient was seronegative by Lyme enzyme-linked immunosorbent assay but showed suspicious bands on Western blot. Findings of a Warthin-Starry stain of a skin biopsy specimen of the eruption revealed a Borrelia-compatible structure. Reinfection was not believed to have occurred. Further treatment with minocycline led to resolution of the erythema migrans."		
54.	**Schutzer SE.**	1993	**Seronegative Lyme disease.**	In "Lyme Disease," ed. P. Coyle, p.192
		"The number and percentage of seronegative Lyme disease cases remain controversial. At some academic centers the estimate is 5%, and in certain private settings the number may be higher. There is little question that seronegative Lyme disease can exist."		
55.	**Sigal LH.**	1993	**Lyme disease: testing and treatment. Who should be tested and treated for Lyme disease**	Rheum Dis Clin North Am, 19(1):79-93
		[From the abstract:] "LD is not a diagnosis that can be made on the basis of serologic testing. By this is meant that vague symptoms plus a positive serologic test do not assure that the patient has LD. On the other hand, a patient with ECM or other manifestations of LD may still be seronegative."		
56.	**Steere AC.**	1993	**Seronegative Lyme disease.**	JAMA, (270):1369.
		"The number and percentage of seronegative Lyme disease cases remain controversial. At some academic centers the estimate is 5%, and in certain private settings the number may be higher. There is little question that seronegative Lyme disease can exist."		
57.	**Preac-Mursic V; Pfister HW; Spiegel H; Burk R; Wilske B; el al.**	1993	**First isolation of Borrelia burgdorferi from an iris biopsy.**	J Clin Neuroophthalmology, 13(3):155-61; discussion 162.
		[From the abstract:] "Antibiotic therapy may abrogate the antibody response to the infection as shown by our results. Patients may have subclinical or clinical disease without diagnostic antibody titers. Persistence of B. burgdorferi cannot be excluded when the serum is negative for antibodies against it."		
58.	**Oksi J; Viljanen MK; Kalimo H; Peltonen R; Marttia R; Salomaa P; et al.**	1993	**Fatal encephalitis caused by concomitant infection with tick-borne encephalitis virus and Borrelia burgdorferi.**	Clinical Infectious Diseases, 16(3):392-6
		"Serology for Borrelia was negative. Autopsy revealed necrotizing encephalitis and myelitis with involvement of the dorsal root ganglion. With use of polymerase chain reaction tests, segments of two separate genes of B. burgdorferi were amplified from the patient's CSF."		

	Author	Year	Title	Journal
59.	**Keller TL; Halperin JJ; Whitman M.**	1992	**PCR detection of Borrelia burgdorferi DNA in cerebrospinal fluid of Lyme neuroborreliosis patients.**	Neurology, 42(1):32-42
			"PCR detected B burgdorferi DNA in the CSF of seven patients whose blood serology ...failed to demonstrate prior exposure to the organism. The failure of existing diagnostic methods to detect patients exhibiting well-recognized manifestations of Lyme disease has been described. This has generally been attributed to abrogation of the host immune response by noncurative antimicrobial treatment. This explanation seems unlikely in at least four of the seven patients who had no recollection of having received antibiotics. "	
60.	**Banyas GT.**	1992	**Difficulties with Lyme serology.**	J Am Optom Assoc, 63(2):135-9
			[From the abstract:] "A major problem has been seronegativity in persons possessing the disease (false negatives). At present, seronegativity in persons strongly suspected of having Lyme disease does not necessarily exclude the diagnosis of Lyme disease. The clinician must recognize this in patients who may have Lyme disease or a recurrence of the disease."	
61.	**Dinerman H; Steere AC.**	1992	**Lyme disease associated with fibromyalgia.**	Annals of Internal Medicine, 117:281-5
			"The small percentage of patients who are seronegative by enzyme-linked immunosorbent assay (ELISA) later in the illness usually have positive Western blots or cellular immune responses to borrelial antigens (9,10)."	
62.	**Fraser DD; Kong LI; Miller FW.**	1992	**Molecular detection of persistent Borrelia burgdorferi in a man with dermatomyositis.**	Clinical & Experimental Rheumatology, 10(4):387-90.
			[From the abstract:] "Antibiotic therapy was reinstituted after Borrelia burgdorferi was detected in the patient's peripheral blood leukocytes by the polymerase chain reaction (PCR). All serologic, T-cell stimulation, and western blot analyses, however, were negative... In addition, this case emphasizes the potential clinical utility of PCR technology in evaluating the persistent sero-negative Lyme disease which may occur in immunocompromised individuals."	
63.	**Keller TL; Halperin JJ; Whitman M.**	1992	**PCR detection of Borrelia burgdorferi DNA in cerebrospinal fluid of Lyme neuroborreliosis patients.**	Neurology, 42(1):32-42
			[Abstract:] "We used the polymerase chain reaction (PCR), a method useful in the detection of Borrelia burgdorferi in vitro, to evaluate CSF in patients thought to have neuroborreliosis. Nested pairs of oligonucleotide primers were designed to recognize the C-terminal region of B burgdorferi OspA. CSF samples were obtained from (1) patients with immunologic evidence of systemic B burgdorferi infection and clinical manifestations suggestive of CNS dysfunction, (2) seronegative patients with clinical disorders consistent with Lyme borreliosis, and (3) patient and contamination controls; all were analyzed in a blinded fashion. PCR detected B burgdorferi OspA DNA in CSF of (1) 10 of 11 patients with Lyme encephalopathy, (2) 28 of 37 patients with inflammatory CNS disease, (3) seven of seven seronegative patients with Lyme-compatible disorders, and (4) zero of 23 patient controls. Zero of 83 additional contamination controls were PCR-positive"	
64.	**Faller J; Thompson F; Hamilton W.**	1991	**PCR detection of Borrelia burgdorferi DNA in cerebrospinal fluid of Lyme neuroborreliosis patients.**	Foot & Ankle, 11(4):236-238.
			"Patient 8 had several negative ELISA assays. She then had a lymphocyte reactivity test for cell mediated immune (CMI) response to Borrelia burgdorferi antigen. Her peripheral blood lymphocytes were markedly responsive to the spirochete, with an index of 46 (18 is three standard deviations above the controls.)"	
65.	**Reik L, Jr.**	1991	**Lyme Disease and the Nervous System.**	New York: Thieme Medical Publishers, Inc.
			"In some cases, specific serum antibody is present but sequestered in immune complexes, and therefore not measurable by routine ELISA." *"...test results for serum antibodies are not always positive when neurologic abnormalities develop, especially in stage 2."*	
66.	**Havlik J; Rohacova H; Hulinska D; et al.**	1991	**Seronegative Lyme borreliosis.**	The 5th European Congress of Clinical Microbiol. and Infect. Diseases, Sept. 8-11, Oslo, Norway:1385.
67.	**Nields JA; Kueton JF.**	1991	**Tullio phenomenon and seronegative Lyme borreliosis.**	Lancet, 338(8759):128-9
68.	**Steere AC.**	1991	**Rheumatology research in the 90s.**	Rheumatology News

	Author	*Year*	*Title*	*Journal*
69.	**Nadelman RB; Pavia CS; Magnarelle LA; Wormser GP.**	1990	**Isolation of Borrelia burgdorferi from the blood of seven patients with Lyme disease.**	American Journal of Medicine, 88:21-6
			"The absence of significant antibody titers to B. burgdorferi is not uncommon in Lyme disease, especially in early disease. ...Although it was initially believed that patients with neurologic Lyme disease generally have antibodies to B. burgdorferi, this may not always be the case. ...We would advise that in an endemic area, the differential diagnosis of nonspecific muscle and joint aches without rash should include Lyme disease--even in the absence of antibodies to B. burgdorferi."	
70.	**Schutzer SE; Coyle PK; Belman AL; Golightly MG; Drulle J.**	1990	**Sequestration of antibody to Borrelia burgdorferi in immune complexes in seronegative Lyme disease.**	Lancet, 335(8685):312-5
			[From the abstract:] "Apparent B burgdorferi seronegativity in serum immune complexes may thus be due to sequestration of antibody in immune complexes."	
71.	**Bianchi G; Rovetta G; Monteforte P; Fumarola D; et al.**	1990	**Articular involvement in European patients with Lyme disease. A report of 32 Italian patients.**	British Journal of Rheumatology, 29(3):178-80
			[From the abstract:] "In addition, interpreting serological tests for antibodies against B. burgdorferi and the real prevalence of arthritis in LD [Lyme disease] is complicated by the possible existence of seronegative LD and by the effect of early antibiotic treatment."	
72.	**Dieterle L; Kubina FG; Staudacher T; Budingen HJ.**	1989	**Neuro-borreliosis or intervertebral disk prolapse?**	Dtsch Med Wochenschr, 114(42):1602-6
			[From the abstract "Between September 1986 and November 1988, 17 patients were hospitalized and treated for neuro-borreliosis. ...Three of 14 patients had no IgG antibodies against Borrelia, either in serum or cerebrospinal fluid at the initial examination, two had positive titres in serum only.	
73.	**Guy EC; Turner AM.**	1989	**Seronegative neuroborreliosis.**	Lancet, 1:441
			"We wish to report a case with a clinical diagnosis of acute Lyme neuroborreliosis for whom negative serology was reported by a Lyme disease referral centre using ELISA. ... *Western blot analysis of sera revealed both IgM and IgG binding to several B burgdorferi proteins, compatible with acute Lyme disease. Antibody binding to a larger number of B burgdorferi proteins was found when the CSF was tested similarly. Our observation of a more diverse antibody response in CSF compared with serum is consistent with the findings of a previous study which showed 44% of patients with neurological manifestations of Lyme disease to have positive CSF antibody titres but negative serum titres when measured by ELISA. Analysis of CSF for the detection of IgM and IgG binding to B burgdorferi is essential if seronegative cases of acute Lyme neuroborreliosis are to be identified."*	
74.	**MacDonald AB.**	1989	**Gestational Lyme borreliosis. Implications for the fetus.**	Rheum Dis Clin North Am, 15(4):657-77
			"From a biologic perspective, most of the fatal cases of LB [Lyme borreliosis] in pregnancy were reactive either in titers in the borderline region or were completely nonreactive in serologic tests. The tendency toward seronegativity in pregnancy makes maternal serology a less satisfactory discriminator of maternal infection and useless as a practical tool to predict the actual state of the fetus..."	
75.	**Trock DH; Craft JE; Rahn DW.**	1989	**Clinical manifestations of Lyme disease in the United States.**	Connecticut Medicine, Vol 53, No. 6
			"...cases of seronegative Lyme disease have been reported..."	

	Author	*Year*	*Title*	*Journal*
76.	**Dattwyler RJ; Volkman DJ; Luft BJ.**	1989	**Immunologic aspects of Lyme borreliosis.**	Rev Infect Dis, II(Suppl 6):S1494-98.
			"Three separate groups of investigators have reported individuals who lacked diagnostic levels of specific antibody in their serum, yet had neurologic involvement and diagnostic levels of antibody in their CSF. We have confirmed this finding in our laboratory."	
77.	**Preac-Mursic V; Weber K; Pfister HW; Wilske B; Gross B; Baumann A; Prokop J.**	1989	**Survival of Borrelia burgdorferi in antibiotically treated patients with Lyme borreliosis.**	Infection, 17(6):355-9
			[From the abstract:] "We conclude that early stage of the disease as well as chronic Lyme disease with persistence of B. burgdorferi after antibiotic therapy cannot be excluded when the serum is negative for antibodies against B. burgdorferi."	
			[Seronegativity:] "As shown, negative antibody-titers do not provide evidence for successful therapy; antibody-titers may become negative despite persistence of B. burgdorferi." (p.358)	
78.	**Berger BW; MacDonald AB; Benach JL.**	1988	**Use of an autologous antigen in the serologic testing of patients with erythema migrans of Lyme disease.**	Journal of the American Academy of Dermatology, 18(6):1243-6
			[Abstract:] "We attempted to detect an early rise in antibody titers to Borrelia burgdorferi in the serum of patients with erythema migrans of Lyme disease by utilizing B. burgdorferi isolates obtained from patients' own skin lesions instead of the B31 reference strain. B. burgdorferi was isolated from nine of 23 skin biopsy specimens submitted for culture. Elevated antibody titers were not detected in any of the 23 acute serum samples by immunofluorescence assay. The antigens derived from patient isolates were no more effective than the reference strain in detecting antibodies in patients with early Lyme disease."	
79.	**Dattwyler RJ; Volkman DJ; Luft BJ; Halperin JJ; Thomas J; Golightly MG.**	1988	**Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte responses to Borrelia burgdorferi.**	New England Journal of Medicine, 1;319(22):1441-6
			[From the abstract:] "Although these patients had clinically active disease, none had diagnostic levels of antibodies to B. burgdorferi on either a standard enzyme-*linked immunosorbent assay or immunoflourescence assy. ...We conclude that the presence of chronic Lyme disease cannot be excluded by the absence of antibodies against B. burgdorferi and that a specific T-cell blastogenic response to B. burgdorferi is evidence of infection in seronegative patients with clinical indications of chronic Lyme disease."*	
80.	**Pachner AR.**	1988	**Borrelia burgdorferi in the nervous system: the new "great imitator".**	Annals of the New York Academy of Sciences, 539:56-64
			"The antibody response in serum in CNS Lyme disease seems to be related to the presence of other manifestations; patients who have had both arthritis and CNS disease have quite high titers, while those with only CNS disease sometimes do not."	
81.	**Lavoie PE; Lattner BP; Duray PH; Barbour AG; Johnson HC.**	1987	**Culture positive seronegative transplacental Lyme borreliosis infant mortality.**	Arthritis Rheum, Vol 30 No 4, 3(Suppl):S50
			"We report a culture positive neonatal death occurring in California, a low endemic region. ...Bb was grown from a frontal cerebral cortex inoculation. The spirochete appeared similar to the original Long Island tick isolate. Silver stain of brain & heart was confirmatory of tissue infection. The mother had been having migratory arthralgias and malaise since experiencing horse fly & mosquito bites while camping on the Maine coast in 1971. The family was seronegative for LB by ELISA at Yale. Cardiolipin antibodies were also not found."	
82.	**MacDonald AB.**	1987	**Lyme disease. A neuro-ophthalmologic view.**	Journal of Clinical Neuro-Ophthalmology, 7(4):185-90
			[From the abstract:] "Potential pitfalls in the diagnosis of Lyme disease with an emphasis on false negative serology and currently available diagnostic modalities are presented."	

	Author	Year	Title	Journal
83.	**Hederstedt B; Hovmark A; Stiernstedt G; Asbring E.**	1986	**[Borrelia-diagnos aktuell aret om visar serologisk undersokning av 1985 ars fall.]**	Lakartidningen, 83:3987-89

[According to Guy & Turner, 1989, this study found that 44% of patients with neurological Lyme disease had positive CSF antibody titres but negative serum titres when measured using ELISA testing.]

84.	**Schmidt R; Kabatzki J; Hartung S; Ackermann R.**	1985	**[Erythema migrans borreliosis in the Federal Republic of Germany. Epidemiology and clinical aspects.]**	Deutsche Medizinische Wochenschrift, 110(47):1803-7

[Abstract:] "A positive antibody titre against Ixodes-ricinus-Borrelia (burgdorferi), using indirect immunofluorescence or ELISA, could be detected in serum and (or) liquor of 935 (32%) out of a total of 2955 patients between January 1984 and July 1985. In 289 of these cases the typical clinical manifestations were lacking whereas a characteristic disease picture enabled a diagnosis to be made in 171 patients with negative or borderline antibody titres. The 1106 cases of infection observed covered all regions of the country. A typical clinical syndrome was seen in 817 (74%) of these. Most common were erythema chronicum migrans (n = 458) and meningopolyneuritis Garin-Bujadoux-Bannwarth (n = 404); in 42% of the cases meningopolyneuritis was preceded by an erythema. Arthritis (n = 63), acrodermatitis chronica atrophicans (n = 72), carditis (n = 13) and lymphadenosis benigna cutis (n = 5) were much less common. Chronic Borrelian encephalomyelitis (n = 45) appeared surprisingly often (n = 45). The fact that in 73% of cases the various syndromes appeared alone, were double in 24% and combined only in 3%, illustrates the polymorphic nature of this disease."

Other Spirochetes

85.	**Stephan C; Hunfeld KP; Schonberg A; et al.**	2000	**[Leptospirosis after a staff outing].**	Dtsch Med Wochenschr, 125(20):623-627.

"[From the abstract:] "The symptoms are non-specific and, moreover, in some cases the laboratory tests are negative, so that clinical diagnosis remains crucial."

86.	**Jacobs R.**	1999	**Infectious Diseases: Spirochetal. Syphilis.**	Current Medical Diagnosis & Treatment 1999. 38th Edition. Appleton & Lange. Stamford, CT. Ed. Tierney LM Jr; McPhee SJ, Papadakis MA.

"The VDRL titer is usually high (>1:32) in secondary syphilis and tends to be lower (< 1:4) or even negative in late forms of syphilis."

87.	**Vecsei AK, Vecsei PV, Dangl-Erlach E,**	1999	**[Congenital syphilis: late diagnosis in spite of screening].**	Wien Klin Wochenschr, 111(10):410-3

[Abstract:] "We report the case of an infant in whom congenital syphilis was diagnosed at the age of 5 weeks. The case is remarkable because of (a) the negative venereal disease laboratory test from the cord blood, (b) the incidental diagnosis of the disease in the fifth week of life, (c) pneumonia alba being one of the symptoms, (d) the occurrence of a mild Jarisch-Herxheimer reaction after initiation of penicillin therapy and (e) the successful treatment of infection related anaemia with recombinant human erythropoietin."

	Author	*Year*	*Title*	*Journal*
88.	**Uribe CS; Garcia FA.**	1998	**[Neurosyphilis and the prozone effect].**	Rev Neurol, 27(160):970-2
	[Abstract:] "INTRODUCTION: Neurosyphilis (NS) is an entity which still frequently presents to our Neurology Department. The prozone phenomenon occurs in approximately 2% of all cases of late primary syphilis or secondary syphilis; we have found no cases described of prozone and neurosyphilis occurring together. CLINICAL CASE: We present the unusual case of a 44 year old patient with NS and dementia PGP (progressive general paralysis). Initially serum VDRL was negative, but in CSF reacted at dilutions of 1:32. When serum VDRL was repeated using dilutions, it was reactive 1:128 and serum FTA was also reactive. The patient was treated with i.v. crystalline penicillin, after which his condition improved. CONCLUSIONS: We wish to draw attention to the possibility that patients with a dementia syndrome and negative serum VDRL may have the prozone phenomenon, and the laboratory should therefore be asked to do serial dilutions."		
89.	**Tikjob G; Russel M; Petersen CS; Gerstoft J; Kobayasi T.**	1991	**Seronegative secondary syphilis in a patient with AIDS: identification of Treponema pallidum in biopsy specimen.**	Journal of the American Academy of Dermatology, 24(3):506-8
90.	**Berkowitz K; Baxi L; Fox HE.**	1990	**False-negative syphilis screening: the prozone phenomenon, nonimmune hydrops, and diagnosis of syphilis during pregnancy.**	Am J Obstet Gynecol, 163(3):975-7
	[From the abstract:] "Recently we encountered four cases of false-negative syphilis serologic results in women who gave birth to infants with congenital syphilis. The false-negative results were caused by the prozone phenomenon. The prozone phenomenon, seen during primary and secondary syphilis, occurs because a higher than optimal amount of antibody in the tested sera prevents the flocculation reaction typifying a positive result in reagin tests. Serum dilution is necessary to make the correct diagnosis. We recommend that for any pregnant woman with apparently negative syphilis serologic results in whom fetal compromise of unknown etiology exists, particularly nonimmune hydrops, nontreponemal testing should be repeated using serum dilutions to prevent a missed diagnosis of syphilis. We further recommend serum dilution as a routine procedure for all pregnant women in areas of high syphilis prevalence."		
91.	**Borisenko KK; Vinokurov IN; Toporovskii LM.**	1989	**[Characteristics of the course of latent forms of syphilis].**	Vestn Dermatol Venerol, (11):25-9
	[Abstract:] "Seven patients with latent syphilis are described, in whom the routine serologic tests (RST) were negative during the first examination and over the course of therapy, and the specific tests (T. pallidum immobilization and immunofluorescence) were repeatedly positive before therapy. Early latent seropositive recurrent forms of syphilis were detected in the majority of these patients' sexual partners. The patients were not administered antisyphilis therapy before. The diagnosis of latent seronegative early syphilis negative in the RST is epidemiologically significant, for it helps timely carry out the necessary treatment and prophylaxis measures to prevent the disease dissemination."		
92.	**Ovcinnikov NM.**	1981	**[Important problems in the serodiagnosis of syphilis.].**	Vestn Dermatol Venerol, 8:22-26
	[According to Mattman L., 1993: "It is thought [by Ovcinnikov] that false negative serological tests for syphilis may be explained because cystic and granule stages of the treponeme have not stimulated antibody reactive with the spirochetal stage."]			
93.	**Il'in II. Pakhomova LV.**	1981	**[Seronegative forms of latent syphilis].**	Vestnik Dermatologii i Venerologii. (1):66-9

	Author	*Year*	*Title*	*Journal*
94.	**Sparling PF.**	1971	**Diagnosis and treatment of syphilis.**	New England Journal of Medicine, 284: 642-653

Some infected patients had negative or equivocal serologic tests for syphilis. “These studies [reviewed] emphasize the fact that late syphilis can occur even if all serologic tests are negative.”

Includes a review of recent [as of 1971] evidence indicating that penicillin treatment is not always curative in patients with late syphilis. "Penicillin therapy of neurosyphilis has not been as effective [as in early syphilis]. Several studies have reported relapses... Clinical progression of symptomatic neurosyphilis is relatively common despite antibiotics." (p.650)

95.	**Ch'ien L. Hathaway BM. Israel CW.**	1970	**Seronegative dementia paralytica: report of a case.**	Journal of Neurology, Neurosurgery & Psychiatry. 33(3):376-80
96.	**Hallock J; Tunnessen WW.**	1968	**Congenital syphilis in an infant of a seronegative mother.**	Obstet Gynecol, 32(3):336-8
97.	**Roitburd MF.**	1968	**[2 cases of seronegativity in secondary syphilis].**	Vestn Dermatol Venerol, 42(8):82-3
98.	**Smith JL.**	1968	**Spirochetes in late seronegative syphilis, despite penicillin therapy.**	Med Times, 96(6):611-23
99.	**Smith JL; Israel CW.**	1967	**Spirochetes in the aqueous humor in seronegative ocular syphilis. Persistence after penicillin therapy.**	Archives of Ophthalmology, 44:474-477

"The purpose of this communication is to report the presence of spirochetes in the aqueous humor both before and after the intramuscular administration of 9,200,000 units of long-acting penicillin for seronegative ocular syphilis. The treponemes were found by the flourescein antibody technique, in which the spirochetes stained with flourescein tagged anti-Treponema pallidum globulin when viewed with ultraviolet microscopy. ...

Spirochetes have now been found in aqueous humor, cerebrospinal fluid, liver, and lymph nodes in several patients with late seronegative syphilis."

100.	**Smith JL; Israel CW.**	1967	**The presence of spirochetes in late seronegative syphilis.**	JAMA, 199(13):980-984

[Abstract:] "Late seronegative syphilis refers to clinical signs of ocular or neurosyphilis in a patient whose routine blood (reagin) test is nonreactive, but in whom a specific treponemal test is reactive. This report documents the presence of spirochetes in aqueous humor, cerebrospinal fluid, and at liver biopsy in such patients. Spirochetes have been found in the aqueous humor with no biomicroscopic abnormality and in cerebrospinal fluids which had normal cell counts, protein levels, and reagin and colloidal gold test results. Identification of the organisms depends on use of the flourescein antibody technique, in that spirochetes stain with flourescein-tagged anti-Treponema pallidum globulin on ultraviolet microscopy."

[From the article:] "The finding of motile spirochetes in the aqueous humor of animal eyes which showed no clinical signs of inflammation at the time of paracentesis led to the initial clinical studies reported here. It must be emphasized that several patients were found to have negative findings in darkfield examinations of aqueous humor and cerebrospinal fluid, in which later study with the fluorescent antibody technique revealed morphologically typical spirochetes which stained with anti-T pallidum globulin. Darkfield examination of CSF requires that the fluid be centrifuged and examined within ten minutes after lumbar puncture in order to see motile treponemes."

Author	*Year*	*Title*	*Journal*
101. **Smith JL; Singer JA; Moore MB, Jr; Yobs AR.**	1965	**Sero-negative ocular and neuro-syphilis.**	American Journal of Ophthalmology, 59:753-762
102. **Taylor WH; Smith JL; Singer JA.**	1965	**Experimental seronegative syphilis.**	American Journal of Ophthalmology, 60:1093-1098
103. **McCord.**	1929	**Study of two hundred autopsies made on syphilitic foetuses.**	Am J Obst & Gynec, 18:597